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CONTRIBUTIONS FROM THE ZOÖLOGICAL LABORATORY OF THE MUSEUM OF COMPARATIVE ZOOLOGY AT HARVARD COLLEGE, UNDER THE DIRECTION OF E. L. MARK. — No. 149.

METABOLISM AND DIVISION IN PROTOZOA.

BY AMOS W. PETERS.

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I. Introduction.

The process of cell-division has been chiefly investigated for the sake of determining the form-changes which characterize it. For this purpose the descriptive-morphological method was found well adapted. The present work deals with a different aspect of the same process and employs a different method. Cell-division, being one of the fundamental powers of organized matter, presents a variety of relations for study. Its morphological aspect has naturally received attention first, but the attempt at further analysis of the results has given rise to a new class of problems. This state of affairs is well summarized by Korschelt und Heider (:02, p. 253), who say: "Wir haben bei der Frage nach den letzten Ursachen der im Vorstehenden beschriebenen Erscheinungen immer auf den uns noch unbekannten Chemismus der lebenden Elemente, auf unbekannte feinere Structuren oder auf den noch nicht ergründeten Mechanismus der Bewegungserscheinungen verweisen müssen." It is from the side of the first mentioned of these unknown, but

not necessarily incompatible, factors, that the present work has been undertaken. In this work the chemical relations in living organisms, especially with reference to their power to divide, have been studied, and the method has of necessity been experimental.

The work was done under the continuous supervision of Professor E. L. Mark, and I take this opportunity to thank him for his valuable and kindly criticism. I am also under much obligation to him for meeting the numerous and varied material needs of these experiments.

The influence on cell-division of *substances* having a relation to it can be best studied experimentally upon free-living cells. Their media afford the important requirement of the experimental method, namely, the ability of the experimenter to vary the conditions. Since there is an active interchange between medium and cell, even the internal conditions of an organism may thus be varied. However, the distinction between external and internal conditions is at best one of not much logical force. The necessity of performing experiments upon the living object *by means of a liquid medium* made the analysis of physico-chemical relations a primary object. No adequate interpretation of the changes of cells in media is possible without this analysis. Whether it is only a preliminary necessity, or whether, if complete, such analysis would constitute the whole interpretation, must be left for the progress of investigation to determine. In this research the object was to push physico-chemical interpretation as far as the present development of physico-chemical methods permits. This part of the work constitutes one of the two essential modes of explanation here attempted. But it soon became evident that in the present state of knowledge this method of interpretation when applied alone accounts only incompletely for the ascertained facts. Hence the adjustment of the organism to a particular combination of conditions was adopted as a complementary, but in itself also incomplete, principle of explanation. This latter principle is elaborated in the concrete case of Stentor in a subsequent section. It is true that the conception of adjustment is an unanalyzed complex, and we do not know whether it expresses more than, or only as much as, the physico-chemical interpretation could if developed to its limit. At present it is a useful means of description, and the two contrasted modes of interpretation are here regarded as of equal *practical* importance. The media of free-living cells always contain salts in solution. Likewise it is shown by chemical analysis that cells contain salts, although their condition in the living organism is not so precisely known. Salts that are normally present in the cell or its medium I shall for convenience term the *physiological salts*.

It is almost entirely to this class of substances — and, it must be added, to a particular phase only of this extensive subject — that my studies have been directed.

Much work has been done upon the relation of salts to animal cells, the most of which bears only an indirect relation to that which is here attempted. In the experiments of mammalian physiology the principal problem has been to find out the function of the salts in *general metabolism*. The history of this subject is well given by Rywosh (:00). In another class of experiments the aim has been to determine what salts are necessary for the *development* of the animal, or what is the influence of salts upon that process. This is well illustrated by the extensive work of Herbst ('93 to '99) upon marine Invertebrates. The Protozoa, the group dealt with in the present paper, have been made the objects of much chemical experimentation. But the relation between the *process of division and salts physiologically present* in organisms or their media has received but little direct investigation. In Protozoa the division-reaction, if so it may be named, has not been treated heretofore in a *numerical* way as a criterion of the comparative effects of the physiological salts, used either singly or in combination. Of interest in the present work, so far as they bear on division, are the experiments upon artificial fertilization with salts, as done by Loeb ('99), Morgan ('99), and Viguier (:01). These questions suggest themselves: In what relation do the salts *normally* stand to the process of division, and is their action osmotic, or chemical, or both? The analogy between the egg-cell and the protozoan has been frequently stated. The present work was suggested by one feature of the mode of differentiation in the embryo. *Unequal rate of division* is seen to be an important factor in determining the relative positions of parts in the embryo. Our ignorance of the special factors involved here is well expressed by Minot (:03, pp. 38–39). "The reason for the unequal growth is unknown. We have not even an hypothesis to offer as to why one group of cells multiplies or expands faster than another group of apparently similar cells close by in the same germ layer. It is no real explanation to say that it is the result of heredity, for that leaves us as completely in the dark as ever as to the physiological factors at work in the developing individual." This question naturally arises: To what extent, if any, is the rapidity of division influenced by *normal* chemical factors, especially the ever present physiological salts? A protozoan was selected as the object because it seemed to present fewer complications with other possible factors than exist in the case of an association of cells.

Most of the following experiments were made upon *Stentor coeruleus*. This object has special advantages for the particular end in view. The animal was found to have a high degree of sensitiveness to its environment. Its responses differed in kind, and especially in degree, with variations in the reagents applied. Both the raising of cultures and the experiments themselves showed that Stentor differs from some other Protozoa in this respect. This fact permitted a much greater variation of the experimental factors than a more uniform responsiveness would have done. The qualities above described apply in its cell-division and are probably due to the close relation between that process and metabolism. The other interesting reactions of the animal were observed only incidentally.

II. General Methods and Technique.

The methods and technique for experimentation upon Protozoa require much consideration on account of the variety of conditions involved. Usually each case requires special treatment, but in the following experiments certain aspects of the subject were of such constant occurrence that they may be dealt with once for all. Only the essential points will be mentioned.

Since these studies are chiefly concerned with metabolic problems, it was necessary to maintain the animals experimented upon under conditions as nearly normal as possible. Hence the first requirement was some mechanical device for retaining Stentors in a given medium and also for transferring them to it. For the former purpose the hanging-drop method was used at first. Since many animals were required, a large number of drops was necessary. Upon one side of a thoroughly cleaned plate of thin glass (the thin glass used for photographic negatives answers the purpose well) were placed numerous drops, each containing from five to eight Stentors. With a quick motion this plate was inverted over a shallow cylindrical glass vessel, half filled with water, the edge of the vessel having been previously smeared with vaseline. The whole was then placed under a dissecting microscope, a rough map of the field with its contained drops was made, and the number of Stentors in each drop written upon the map. When, after experimentation, the next count was made, the record was continued upon the same map. With a little practice it became easy to regulate approximately by the size of the pipette used the number of Stentors put into a drop. This method was practicable and expeditious for handling large numbers of individuals and afforded excellent opportunity for any desired examination with ob-

jectives of low power. It was finally rejected, perhaps unnecessarily, for the method next described in consideration of the fact that conditions in a drop might not be entirely normal for so large an animal as Stentor. For almost all other Protozoa this objection would not hold.

Solid watch-glasses were then adopted; these were polished so as to fit closely when piled on top of one another. They were shallow, about 50 mm. in diameter and 10 mm. deep, and had concave internal surfaces, thus presenting no angles in which the animals might lodge and so escape observation and counting. A watch-glass was only partially filled with the required medium, 2 to 4 cc. being placed in each. No more animals were introduced than could be conveniently counted under a dissecting microscope. When piled up the watch-glasses formed a series of closed chambers with sufficient air space and with a much larger proportion of liquid to each Stentor than in the hanging-drop method. As nearly as could be imitated the conditions of a minute natural pond existed here. This method was used for nearly all experiments.

An important point in any method is the transference of the organisms from their native culture medium to the test-medium in the observation glass. To avoid contamination of the latter, or to reduce it to an infinitesimal amount, the following practice was adopted. A considerable number of Stentors were taken from their usual location upon one side of the culture jar with a medicine dropper and placed in a watch, glass (No. 1). Soon these would gather together in one part of the vessel on account of the presence there of zoögloea, the particular direction of the light, etc. With as small a quantity of liquid as possible, they were then transferred to watch-glass No. 2, which contained a considerable quantity of the medium to be used for the given experiment. In the same way, except that pipettes of smaller bore and a dissecting microscope were now used, they were repeatedly transferred to successive "ponds" of the required medium. From the last watch-glass "pond" a few at a time were transferred with a capillary pipette to the test watch-glass containing the pure medium. This I call the method of multiple transference.

How many such transfers are necessary? Fortunately there is a good test to determine this point. When the electrical conductivity of the medium — using electrodes that can be dipped into the liquid of a watch-glass and then removed — shows no *immediate* change upon the introduction of Stentors, then there is no contamination. In the case of multiple transference from culture liquid to distilled water, it was

determined that from two to four transfers were sufficient to prevent any perceptible contamination. The number necessary depends much upon the experimenter's personal method of working. The construction of the pipettes is also an important factor. It appears from the following consideration that the amount of contamination resulting upon the dilution following the use of the pipette must be exceedingly small. Capillary pipettes can be made of such small diameters as to admit but one Stentor at a time, and under the magnifier five to ten animals can be drawn in with a very small quantity of water. This small amount of liquid is furthermore a very small proportion of the 4 to 5 cc. with which it is diluted in the next watch-glass. It follows that successive transfers rapidly multiply the dilution of the original contamination, which at length becomes infinitesimal. This source of matter foreign to the desired medium can be reduced to as low a value as that resulting from the practically unavoidable contact with air and glass. The subject of the solubility of glass will be treated of in the experiments where that factor becomes of importance in drawing conclusions.

The selection of the Stentors for experiment is also a matter of much importance. No other factor that might be overlooked would so quickly bring discrepant results. If the metabolic effects of different reagents are to be compared, it is important that the experiments be conducted, if possible, upon animals in the same condition. When that is not practicable, it is important to have a means of comparison by which different results can be reduced to a common standard. The following experiments offer illustration of both procedures. The experimenter who maintains a continuous supply of material (Stentor) will have on hand cultures in various stages of development. *For comparative experiments the animals should be taken invariably from the same culture.* A good, well-established culture has considerable longevity and makes the above rule practicable. The results are still more favorable when the experimenter has learned to raise healthy cultures by a uniform method. In any case Stentors presenting abnormal or special conditions, such as the undersized, the pale, those that are conjugating or dividing, should be rejected. Material should always be abundant, for if it is, practice soon secures a very uniform selection of normal animals. Among the examples which follow there are some which show the possible reduction of results from different sources to a common metabolic standard.

The question as to the number of organisms that are necessary to a given experiment is one that cannot be answered by a general statement. In experiments upon metabolism where division is the criterion, cumula-

tive experience with the animal's normal condition and its reaction to various substances aids in forming a judgment as to the numbers to be used. Before one knows the normal adjustments of the animal, larger numbers and more repetition may be necessary than later in the course of the work. The present experiments were often so planned either that each involved the one preceding it, or else that the new experiment was based upon the assumption of the validity of those preceding, and so led to discrepancy if the assumption was false. The above discussion applies to a certain class of experiments the validity of which is but little increased by repetition, but such are not numerous in biology.

The animals were segregated into groups, five to ten being placed in a watch-glass and constituting a preparation. This was a convenient number, for it was possible to count them under the dissecting microscope even after multiplication had taken place. The condition after twenty-four to forty-eight hours, usually the latter interval, was regarded as the one most representative of the effects of the medium upon division. Experiment shows that, in general, division keeps pace with metabolism, the latter of course depending upon the supply of food. In experiments with liquid media it is necessary to exclude every form of natural food, if it be desired to study the effect of the pure medium consisting, e. g., of a salt solution. Hence the Stentors in these experiments carried on their metabolic processes at the expense of the reserve nutriment which they contained when taken from their original culture. Consequently the effects upon division have to be observed early in the period of their subjection to a medium practically free from food. Some results observed after long intervals show that Stentor can live a long time without other food than physiological salts, but the process of multiplication always had its maximum in about the initial twenty-four to forty-eight hours. Sometimes it was necessary for a special purpose to take results after still shorter intervals.

The question whether division occurs in cycles has an important bearing upon any method of investigation whose results are based upon counting the number of organisms after a period of division. If there is such periodicity, it would be important to know whether the animals taken for comparative experiments had been taken in the maximum or in the mimimum of the cycle. In the case of Stentor neither direct observation nor the experiments made, furnish evidence of any *inherent* periodicity of division. The present experiments show that, except when some special modification of the medium exists (e. g. presence of potassium chloride in excess), multiplication runs, in the main, parallel to meta-

bolism. Furthermore, while the supply of food in cultures and the constitution of the medium, especially with reference to its salt content, are normal, no maxima and mimima of division occur. This condition never lasts indefinitely, and both observation and chemical examination show that there is a definite course of changes natural to cultures from the period of their beginning with a food supply to that of its exhaustion. In an undisturbed culture the decline of Stentor as well as its initial rapid multiplication is due to metabolic conditions. In the present experiments old cultures were frequently revived, and rapidly multiplying ones were sometimes brought to sudden decline, by an apparently slight change in the nutritive conditions of the medium. The experiments on salt media described in this work show the pronounced sensitiveness of these animals to this factor. The response of Stentor to these conditions points to *metabolic exigency* as the efficient factor. To demonstrate experimentally, i. e. by more efficient means than observation of cultures, that periodicity of division occurs in a protozoan, two conditions at least must be fulfilled. First, the experimenter must know the exact composition with reference to salts, food, etc., of the media to which he subjects the animals. Such promiscuous mixtures as hay infusion *of unknown composition* will not suffice. Since frequent chemical analyses are impracticable, it will be necessary to construct by trial artificial media of known composition. The second requirement is that the experimenter should determine the normal adjustments of the animal to the different factors of the proposed media. Without this knowledge it is impossible to ascertain whether observed increase and decrease of multiplication is due to simple metabolic exigency or to an inherent tendency to cyclic division. A beginning towards meeting both requirements above indicated has been made for Stentor by experiments subsequently described.

In the preparation of reagents such precautions were taken as were appropriate to the end in view, and hence varied somewhat in different experiments. In general this part of the work was made to rest as much as possible upon objective demonstration. Reagents were either tested, even though their labels might be those of reliable manufacturers, or they were prepared personally. The measures used for volumetric purposes were calibrated by means of weighed quantities of liquid, using Sutton's ('96, p. 25) table for air weighing with brass weights.

Standard solutions were carefully made, and consistency among dilute solutions, chlorides, etc., maintained. Dilutions with water from an originally strong solution were made by weight with reference to the

temperature standard. Perhaps distilled water was the reagent demanding most attention. In testing waters of all sorts, as well as culture liquids and reagents in special cases, the method of electrical conductivity was applied. For this purpose the apparatus devised by Nernst ('94) and modified by Maltby ('95) for measuring conductivity was found most useful. For its construction and use the original descriptions must be consulted.

For biological purposes a great number of test-cells of various constructions was found necessary. Variation in the size and distance apart of the electrodes was indispensable in some critical experiments where the use of platinized electrodes was not allowable. On this account and owing to the good qualities of the apparatus itself for the avoidance of polarization, it was never found necessary to use platinized electrodes in the test-cells. For convenience in the subsequent description of experiments I shall adopt the same notation for the measuring tubes of the instruments as are given to them by Maltby ('94, Fig. 4, p. 142). The tubes most referred to there are on the right-hand side, the larger one being marked W″ and the smaller W.

III. Conditions of Growth in Stentor, and the Management of Cultures.

For my study it was soon found desirable to maintain a continuous supply of vigorous material. *Later* it became evident that it was still more necessary to know the origin of the Stentors used. These requirements led to the artificial culture of Infusoria in the laboratory, instead of the continual acquisition of new material from ponds in the vicinity. Artificial cultures incidentally exhibit some otherwise unnoticed aspects of the metabolism of Stentor, and are a valuable means of confirming by mass-culture the results of special experiments. Such cultures furnish to observation, and especially to chemical tests, much indication of the continuous change in constitution taking place in a medium and of the phases of these changes to which the different kinds of Infusoria are respectively adjusted. But the practice of making artificial mass-cultures had the disadvantage of consuming much time. Numerous empirical trials had to be made before sufficient insight was obtained into the conditions to which Stentors adjust themselves. This animal was observed to differ from some other Infusoria (e.g., Paramaecium) in having a more circumscribed range of favorable conditions. The salt media devised as the result of successive experiments, and described in the sections treat-

ing of adjustment, furnished the most successful starting points for mass-culture.

The original cultures were obtained by collecting a variety of solid material from the edge of ponds in the vicinity of Cambridge. The collection must not be too rich in fermentable matter. If this precaution is not observed, such Stentors as are brought in with it will be killed off by an excess of the initial fermentation characteristic of every culture. Brown leaves and dead reeds were found most useful. Both in artificial and in natural cultures *decaying cellulose proved to be the best source of the food supply for Stentor.* It is of course only the source, not the food itself. An excess of fleshy green matter must be avoided, but some thread algae may be included. No more pond water need be taken than is necessary to cover the collection. It is best to transfer the solid matter to a jar while the latter is in the water. It was customary to set mass-cultures, whether natural or artificial, in cylindrical glass jars of about 4000 cc. capacity. The object in using such large vessels was to obtain cultures whose longevity would be great in proportion to the food supply. To each jar was transferred enough of the collected matter to occupy about one tenth of its volume. The jar was then filled with tap water and kept at room temperature, or if convenient a little warmer. Jars should never be allowed to cool to low temperature, should be covered with a pane of glass to prevent evaporation, and should stand in diffuse daylight. Provided Stentors are originally present, some of these cultures will be successful, and after one or two weeks will show a growth of Stentors localized on the side of the jar away from the light. If too much fermentable material was present, it will take longer for the culture to develop, because Stentors will not appear in abundance until after the initial fermentation has ceased. In some cases a culture develops after weeks of standing. The above procedure, although frequently successful, carried with it no certainty of result and hence pointed to the necessity of a further study of conditions. Cultures were set with an abundant supply of oxygen-producing water plants, in addition to the leaves and reeds above mentioned. The result was signal failure. This fact, coupled with the observation that many successful cultures contained no evident abundance of algae, shows that the experimenter need take no special precautions to supply oxygen to a Stentor culture. The decaying algae frequently noticed in good cultures probably acted as a source of food.

Observations were made to determine directly the immediate food of Stentor. The animals were compressed under a thin cover glass by gradually withdrawing the liquid medium with filter paper. Their

movements were thus stopped and they were pressed flat. Finally the contents of the cell escaped. Unidentified unicellular vegetable matter of low organization, Euglenae, and Arcellae were the most frequent kinds of food present. These organisms were abundantly consumed. Occasionally a Stentor was observed in the act of ingesting a Paramaecium. Although Stentor is omniverous, this fact was of not much avail in raising a culture. Very often it was observed that an *aborting* culture with enough Stentors still present to make a successful start contained an abundance of the direct foods upon which the animals could easily subsist. This observation was repeated so often that it was quite evident that some other important condition than a supply of food had not been fulfilled.

Gradually it became clear that *no other single condition is of such determining influence for free-living cells as the salt content of their liquid medium.* The special experiments, subsequently described, made during the period of these culture experiments, furnished direct evidence for the above proposition. Hence attention was turned to the determination of a favorable salt medium for Stentor. The failures and the partial successes that attended this effort will not all be recorded here. They were instructive in showing that the determination of an animal's adjustments to substances brings to light a metabolic status not so well exhibited by any other method.

The most successful salt medium for raising cultures was obtained by the experiments described in the section on Single and Combined Salts (p. 497). Special tests upon the most successful medium are there described. It consisted of the following combination of salts dissolved in water:

$CaCl_2$	.00055 m.
$NaNO_3$	.00015 m.
$MgSO_4$	.00015 m.
K_2HPO_4	.00015 m.

The sum total of its equivalent concentration is 100 molecular parts in 100,000. The most important constituents are $CaCl_2$ and K_2HPO_4, and the proportions, especially high for $CaCl_2$, are important. As subsequent experiments show, Stentor can live a long time in a favorable salt solution, but multiplication soon ceases. To maintain this, a food supply must be added to the salt solution, and this requirement has proved to be a difficulty. For the addition of any food that has been found available utterly changes the salt content both qualitatively and

in its proportions. The effect of this upon the animals has been partially overcome by doubling or even trebling the concentration of the above formula so that, if added to distilled water, it would have represented 200 to 300 molecular parts in 100,000. The *excess* of constituents known to be favorable was relied upon to nullify, in part at least, the disturbance of balance due to the addition of food. The same observation applies to the use of tap-water, which was customary for mass cultures.

Having added to about 3500 cc. of tap-water, contained in a 4000 cc. jar, sufficient of the above salts to make the required concentration when the jar is full, the next step was the addition of some dry leaves or dead reeds or both. The smaller the amount the more quickly will the culture develop, but when the supply is too scanty the culture cannot become long-lived. Sometimes it is desirable to heat the leaves and reeds to boiling and then to add the cooled mixture to the salt solution. This causes the solid material to sink immediately, thus facilitating observation at the upper part of the jar, and it also hastens the chemical changes that must precede the rapid growth of Stentor. The final step is to "seed" this culture with a mixture of all sorts of Infusoria and other living material from thriving cultures, including, of course, a large proportion of Stentor. If good cultures are on hand, it is advisable to stir up one of them and transfer about 500 cc. of its liquid to the new culture. If the new culture was successful, it showed the previously described localized growth of Stentors at some time within a week after it had been started. Among the various foods tried was a decoction of barley made by boiling a few grams for about one-half hour. The liquid, containing many particles in suspension, was poured off from the barley grains. When this was very largely diluted with tap-water and seeded, many Infusoria, especially Vorticellae, grew well, but no abundant culture of Stentor could be so raised. The results were somewhat better when the extract of barley was added to the salt medium mentioned above. Tests made independently of the cultures showed that the Stentors did not use as food the particles obtained by boiling the grain. But no other medium equalled this extract in the rapidity of the divisions at first produced. The Stentors became smaller without subsequent growth. Evidently the effect produced was stimulation, not feeding.

The medium probably had a favorable salt constitution, derived from the grain, and it had comparatively little power of fermentation. The classical hay infusion was also tried. While Paramaecia and many other Infusoria grew abundantly in cultures set with hay, Stentor uniformly failed to produce successful cultures. When a hay infusion

became very old, so that it was reduced to the condition of a decaying cellulose medium, it frequently produced Stentors. Likewise when the amount of hay put into the above salt solution was very sparing a transient culture might result. A series of chemical determinations was made upon hay infusion and upon other media for comparison. The results showed that hay furnished so large an amount of fermentable matter as to produce too much acidity in a medium intended for Stentor. This fact also explains the observations upon the age of a hay infusion referred to above, and the amount of hay that may permit a growth of Stentors.

Some operations will now be described that are useful for improving poor cultures or for reviving old ones. Some account will also be given of chemical estimations by the volumetric method that were made in order to compare cultures in different stages. The latter observations were found to furnish a good index of the conditions prevailing in the culture liquid. Only the estimation of acidity, the most useful of these determinations, will be described. Operating upon 5 cc. of the culture liquid I determined the amount of 0.01 normal acid (hydrochloric) or alkali (sodic hydrate or calcic hydrate) required to produce reaction with methyl orange and phenolthalein respectively. The results were compared and other estimations also were made. By making a series of observations upon a single culture from the time of setting it, some idea was gained of its progressive chemical changes. Experience showed that the titration with phenolthalein was the most instructive and the only one necessary. This indicator has the advantage for our purpose of being useful in the titration of carbonic acid and organic acids. These come especially into play in fermenting cultures. In these estimations it was important not to deprive the culture of much liquid, as refilling it disturbed the naturally prevailing conditions. Yet repeated tests were required, and so for this reason the small quantity of 5 cc. was invariably adopted for a test. In the titration of carbonic acid with 0.01 normal alkali (usually sodic hydrate) much difficulty was at first experienced owing to the absorption of this gas from the atmosphere. Hence constant results could not be obtained in successive estimations of the same liquid. The following procedure was devised to overcome the difficulty. The liquid (5 cc.) to be titrated was transferred with a pipette from the culture jar to a short thick-walled test-tube about one cm. in diameter. A few cubic centimeters of kerosene were then poured in to cover the liquid and so prevent absorption of CO_2 by the alkali introduced for titration. Phenolthalein was added. A glass rod was also kept in the tube for stirring. The titration was performed over a white tile. If neces-

sary, the burette could be made to deliver its reagent under the layer of kerosene, by means of tubing of rubber and glass. But without this additional precaution consistent results were easily obtained and the acidity of different cultures or of the same culture from day to day could be compared. Of course carbonic acid was not the only acid which was estimated in the above process. The end reaction took place when the first trace of normal carbonate or of excess of sodic hydroxide was present.

By means of this process the following observations were made. If separate hay and leaf cultures be set with a salt solution as previously described, the acidity of 5 cc. at the beginning may be zero or perhaps equivalent to 0.1 cc. of 0.01 normal sodic hydroxide. After one day an acidity of several tenths will have been reached by both cultures, but the hay culture has the greater acidity. After two to three days the acidity of 5 cc. of the hay cultures was usually equivalent to approximately 0.7 cc. of 0.01 normal sodic hydroxide. After the third day there was no important increase of acidity unless too much fermentable matter had been added. Observation of the Stentors originally placed in these cultures for seed showed that when the acidity exceeded an equivalency of 1 cc. of 0.01 normal sodic hydroxide they became pale, rested upon the *bottom* of the jar, and diminished in numbers. From the bottom of the hay culture their disintegrating forms could be taken and examined. A further examination of successful and unsuccessful cultures showed that the former were either neutral or had an acidity of a few tenths only, whereas the latter were nearly always characterized by a considerable degree of acidity. The fact that heating any of the culture liquids up to the boiling point greatly diminishes their acidity, often indeed producing a condition that gives an akaline reaction with phenolthalein, points to the presence of CO_2, produced by fermentation. As is well known, organic acids also are developed in this process, but in smaller proportions. Soon after the condition of maximum acidity is reached, a covering of Stentors appears over the surface of the culture. In a successful culture the increase in Stentors, Paramaecia, and some other Protozoa may have already reached a great abundance before this condition is attained. Multiplication of Stentors was never observed in an actively fermenting liquid. Observation both of natural and experimental cultures shows that *Stentor is an animal that has adjusted itself to such conditions as prevail in a late stage of a fermenting liquid.* To raise Stentors in a salt solution, it is necessary to reduce to the minimum the intensity of the fermentation inherent in the food supply. This fermen-

tation process is, however, indispensable, for it is the source, direct or indirect, of all the nutriment of Infusoria. Success sometimes attended the introduction of Stentors into a liquid that had completed its active fermentation.

An excellent method of removing the excess of CO_2 and noxious gases from a culture, whether new or old, is to transmit a current of air through it for some time. By reason of the comparatively high partial pressures of the gases in contact with the air, either at the surface or deeper, the whole liquid loses much of these undesirable constituents. This removal of gases is probably of as much importance to the Infusoria as the accompanying increase in oxygen. Unless the odor and other conditions of a culture indicated the need of such aeration, it was found that a growing culture could be ruined by this process. In all cases an estimation of acidity should be made. For some time the practice was resorted to of precipitating the CO_2 as calcium carbonate by the addition of calcic hydroxide. Neutrality or a slight excess of calcic hydroxide are not unfavorable to Stentor. The former condition cannot, of course, be maintained in a culture liquid. This process is inferior to aeration and was abandoned in favor of the latter.

If a culture is old and declining, it is sometimes easier to revive it than to start a new one. This is probably due to the fact that the liquid of a former thriving culture is a favorable salt medium for Stentor. Aeration together with the addition of some brown leaves or dried reeds frequently restored a culture that had run its course. If the liquid was not already laden with salts, it was often found advantageous to add 100 to 200 molecular parts in 100,000 of the usual salts. Such addition of salts was extensively practised, but to what limit it could be carried successfully was never determined. By the use of any or all of the preceding methods, when the conditions suggested them, the same cultures were maintained for months without emptying the contents of the jar.

IV. Acceleration of Division.

My earlier direct experiments upon the rate of cell-division were suggested by the well-known examples of artificial stimulation produced by the treatment of unfertilized eggs, for a short time only, with chemicals. I refer to the experiments of Tichomiroff ('86), O. und R. Hertwig ('87), Morgan ('99), Loeb ('99), Winkler (:00), and others. Among the substances so used occur the physiological salts, — the class in which we are especially interested, — as well as acids, alkalis, extracts of physio-

logically active substances, and alkaloids. A possible analogy suggested itself between the artificially stimulated egg, beginning and continuing its cleavages after an initial impulse, and a possible acceleration in the rate of division in Stentor after similar treatment. I set the question, "Can a temporary impulse due to a substance accelerate the *rate* of division in Stentor or Paramaecium?" The first two of the following experiments were made with this question in view and gave an affirmative answer. It seemed reasonable that the same effects might be kept up by the continuous application of the same substance, and such proved to be the case. I therefore describe these experiments, among others made with a different aim, because they are interesting from another point of view. The comparison of the reaction to a temporarily applied substance with one continuously applied will make clearer the mode of action in the former case. The experiments whose main result was the acceleration of division in Stentor and Paramaecium were as follows.

The Specific Action of Potassic Chloride on Stentor.

Expt. No. 6, Oct. 21, 1901.

For the purpose of applying a liquid medium for a limited time, and to enable me both to apply and to withdraw the medium rapidly, I made use of the apparatus I have elsewhere (Peters, :01) described as a U-cell. This was, essentially, two slides, between which there was placed, in U-form, a properly selected piece of yarn. This cell was placed in an inclined position, the open end of the U uppermost, in a vessel containing enough liquid to permit the immersion of the lower part or the whole of the cell when desirable. By this arrangement liquid can be passed through the wall of the cell without the production of excessive downward pressure upon the contained organisms. The cell was filled with 0.05 m. potassic chloride by means of a capillary pipette, and into it were introduced a considerable number of Stentors, all from the same culture. To this solution the organisms were subjected for ten minutes. During the next ten minutes tap-water was passed through the cell to remove the potassic chloride, and the Stentors were then transferred to a glass vessel containing about 18 cc. of their original culture medium free from other Stentors. In this medium they were mounted in hanging drops, as described in this paper under General Methods and Technique (p. 444). The results are shown in the following table. The time stated is reckoned from the beginning of the experiment. The Mean Result expresses the number to which *on the average* one Stentor increased or diminished.

Time	0 da.	1 da.	3 da.
No. of Stentors	157	202	210
No. of Divisions		?	64
Mortality		?	3
Mean Result		1.29	1.39

A control experiment was made with a similar U-cell in which the Stentors were treated merely with tap-water for twenty minutes, a period of time equivalent to that of the test experiment. The animals were then transferred, as before, to a portion of their native culture medium, free from other Stentors, and mounted in hanging drops. These preparations were set away simultaneously with the test preparations and were kept under the same room conditions. The only essential difference of which I am aware between test and control experiments was the temporary treatment of the former with 0.05 m. potassic chloride. The control gave the following results:

Time	0 da.	1 da.	3 da.
No. of Stentors	188	216	233
No. of Divisions			48
Mortality			4
Mean Result		1.15	1.24

Expt. No. 7, Oct. 24, 1901.

Test experiment similar to Experiment No. 6. Culture medium made to contain 0.05 m. potassic chloride applied to Stentors for ten minutes.

Time	0 da.	1 da.	2 da.
No. of Stentors	212	233	231
No. of Divisions			23
Mortality			3
Mean Result		1.10—	1.09

The control made as described under Experiment No. 6, yielded the following results:

Time	0 da.	1 da.	2 da.
No. of Stentors	139	140	142
No. of Divisions			8
Mortality			6
Mean Result		1.01—	1.02—

Expt. No. 9, Oct. 24, 1901,

was similar to Experiments 6 and 7, except that 0.016 m. potassic chloride was used in the test experiment and that the count was taken after sixteen hours. Test and control contained 142 and 170 Stentors respectively and gave average increases of 1.09 and 1.05 respectively.

I now abandoned the method of temporary application of the reagent, for reasons that will be stated in the subsequent discussion. Hence in all the subsequent tables of this section the given concentration of the reagent was *continuously* applied, except when the time is expressly stated. I also desired to compare the reaction to potassic chloride with that to sodic chloride.

Expt. No. 11, Nov. 1, 1901.

The method of hanging drops was used as previously described. I here adopted the plan, later abandoned, of making both test and control experiments in a medium consisting of diluted and more or less fermented hay infusion. Both media when completed contained the same proportion of strong hay infusion, the dilution being made with tap water. They differed in the fact that one portion of test medium contained potassic chloride added in the proportion of 0.01 m., while the other contained sodic chloride added in the proportion of 0.01 m. The results of three sets of simultaneous preparations were as follows:

	0.01 m. KCl.		0.01 m. NaCl.		Control.	
Time	0 hr.	24 hr.	0 hr.	24 hr.	0 hr.	24 hr.
No. of Stentors . .	247	350	175	209	118	157
No. of Divisions .		106		43		46
Mortality		7		1		2
Mean Result . . .		1.42		1.19		1.33

The Division-Reaction of Stentor to Potassic Chloride.

I shall now describe the phenomenon of division in a potassic chloride medium, which occurred abundantly in this and the following experiments. I observed in the hanging drops of the potassic chloride medium numerous Stentors of sizes ranging from that of a normal individual to that of one so very much smaller than normal, that it was difficult to decide whether to count each as a single individual. The count given above under 0.01 m. potassic chloride does not include all the individuals

that might have been counted, but only the larger ones. In all the applications of chemicals to Protozoa during the course of the present investigation, I have never observed this potassic chloride reaction to occur in any Protozoan except Stentor, nor with any other substance than potassic chloride; neither does it occur in Stentor under normal conditions. When the test medium for drop-preparations of Stentor was made to contain, besides its normal salts, from .01 m. to 0.0167 m. potassic chloride, and the results were compared with the control preparations lacking the potassic chloride, the effect of the reagent was unmistakable. With the greater of the two proportions above mentioned, the effect began to appear sooner than with the less. The formation of new Stentors was in a portion of the cases apparently normal, as judged by the normal appearance of the process and by the size of the new Stentors; but in other cases one or the other of the following abnormal methods prevailed. In normal division the attachment of the anterior of the two new organisms to the posterior one is at some point of the *periphery* of the frontal area of the latter. In one of the abnormal methods there resulted a dumb-bell shaped figure in which the attachment was at the *middle* of this area. In these cases there was a gradual constriction extending with equal rapidity from all sides. A wrinkled appearance of the pellicula was usually evident at the narrowest part of the constriction. In the other abnormal method the process had exactly the appearance of budding, a globular protrusion being gradually constricted off at a point not far behind the adoral band. Many of the new Stentors originating by either method, including both large and small sizes, were of globular form. In view of the possibility that these might be simply portions of extruded cytoplasm, I observed them further. Upon closer examination I rarely failed to find in them one or more of the beads of the nucleus. An adoral band of cilia was not present at first, but grew while the individual was taking on the typical conical form of a normal Stentor; this was true of the dwarfs as well as of those of normal size. To test the vitality of these small Stentors, individuals were removed from the drops in which they occurred, examined for the presence of a nucleus, and then placed in a separate preparation in a medium of hay infusion. This test showed a considerable degree of mortality in such dwarf Stentors, but also yielded numerous apparently perfect, though dwarf, animals. Owing to lack of a proper food-containing medium their further history was not pursued to determine whether they would grow to full size. I regard their growth to full size as highly probable. The above account has been confined as much as possible to a description of

observations, and a discussion of the results will be reserved until the close of the section.

Experience having taught that Stentors are very sensitive and responsive animals, I decided to make an experiment by which to determine whether the numerous Stentors produced in the manner last described were really due to the influence of potassic chloride upon division. The possibility of this suggested itself, and indeed the following experiment was made, before the above-described detailed examination of the process was undertaken.

Expt. No. 13, Nov. 4, 1901.

Preparations of Stentor by the drop method in a 0.0167 m. potassic chloride medium and the control preparations made in hay infusion were strictly parallel in origin of animals used, in time, in dilution of reagent, and in room conditions. They were all handled with great caution to avoid violence, especially in mixing fluids. The following results were obtained:

	0.0167 m. KCl.		Control.	
Time	0 hr.	6 hr.	0 hr.	6 hr.
No. of Stentors	298	331	192	193
No. of Divisions		33		6
Mortality		0		5
Mean Result		1.11		1.00

As in Experiment No. 11, the number recorded in the potassic chloride preparations did not include all that might have been counted, some having been omitted because of their small size. In the control experiment no such difficulty arose, as the offspring were all of nearly normal size.

A comparison of the division-reaction of different Protozoa subjected to the same essential conditions is of interest. I therefore give, briefly, the results of some experiments made with potassic chloride and sodic chloride upon *Paramaecia*. The conditions were parallel with those employed in the experiments on Stentor.

Expt. No. 20, Nov. 20, 1901.

Hanging-drop preparations of Paramaecia in hay infusion were treated for one minute with a 0.167 m. sodic chloride solution. It was

previously determined that a fraction of a minute longer than this was sufficiently injurious to stop locomotion.

	0.167 m. NaCl, 1 min.		Control.	
Time	0 hr.	24 hr.	0 hr.	24 hr.
No. of Paramaecia	39	135	55	205
No. of Divisions		96		150
Mortality		0		0
Mean Result		3.46		3.73

On account of their motion the animals were too numerous to be counted alive, and they were therefore killed before counting.

Expt. No. 21, Nov. 21, 1901.

Purpose and method the same as in the preceding experiment. Paramaecia.

	0.167 m. NaCl, 1 min.		Control.	
Time	0 hr.	30 hr.	0 hr.	30 hr.
No. of Paramaecia	37	49	40	64
No. of Divisions		13		24
Mortality		1		0
Mean Result		1.32		1.60

Expt. No. 10, Oct. 30, 1901.

Hanging-drop preparations 0.01 m. potassic chloride continuously applied in hay infusion. Paramaecia.

	0.01 m. KCl.		Control.	
Time	0 da.	1 da.	0 da.	1 da.
No. of Paramaecia	99	103	87	91
No. of Divisions		4		4
Mortality		0		0
Mean Result		1.04		1.04

Expt. No. 19, Nov. 18, 1901.

Hanging-drop preparations of Paramaecia in hay infusion containing 0.0167 m. potassic chloride.

	0.0167 m. KCl.		Control.	
Time	0 hr.	16 hr.	0 hr.	16 hr.
No. of Paramaecia	59	40	47	45
No. of Divisions		0		0
Mortality		19		2
Mean Result		0.67		0.95

Expt. No. 29, Dec. 7, 1901.

Hanging-drop preparations of Paramaecia in 0.02 m., and 0.01 m., potassic chloride medium, also containing 20 per cent of strong, fermented hay infusion. Medium of control experiment consisted of similar 20 per cent hay infusion only.

	0.02 m. KCl.		0.01 m KCl.		Control.	
Time	0 hr.	42 hr.	0 hr.	24 hr.	0 hr.	42 hr.
No. of Paramaecia	24	23	24	75	24	372
No. of Divisions		2		51		348
Mortality		3		0		0
Mean Result		0.96		3.12		15.50

A number of experiments were made to ascertain the effect of *chloroform* upon the rate of division in Paramaecia. The general result of this was to accelerate division when the reagent was present in the proportion of 5 per cent of a saturated aqueous solution. A concentration of 10 per cent had a retarding influence. I shall give but one experiment.

Expt. No. 22, Nov. 22, 1901.

A sufficient volume of a saturated solution of chloroform in tap-water was added to fermented hay infusion (bacterial food) to make the mixture contain 5 per cent of the strong hay infusion. The control consisted of this diluted 5 per cent hay infusion only. All necessary dilutions were made with tap-water.

	5 % Aq. Chloroform.		0.0167 m. KCl.		Control.	
Time	0 hr.	30 hr.	0 hr.	30 hr.	0 hr.	30 hr.
No. of Paramaecia	53	405	59	226	53	310
No. of divisions		352		166		257
Mortality		0		0		0
Mean result		7.64		3.81		5.85

Summary of Observations.

No. of Expt.	KCl m.	NaCl m.	Chloroform.	Mean Results. Test.	Mean Results. Control.
			STENTOR.		
6	0.05			1.39	1.24
7	0.05			1.09	1.02
9	0.0167			1.09	1.05
11	0.01			1.42	1.33
13	0.0167			1.11	1.00
11		0.0167		1.19	1.33
			PARAMAECIUM.		
20		0.0167		3.46	3.73
21		0.0167		1.32	1.60
10	0.01			1.04	1.04
19	0.0167			0.67	0.95
29	0.02			0.96	15.50
29	0.02			3.12	15.50
22			0.05 Aq.	7.64	5.85
22	0.0167			3.81	5.85

The general results of the experiments described in this section are as follows:

1. Division in Stentor can be accelerated in rate and modified in character by the presence of an excess of potassic chloride in an otherwise normal medium.

2. Division in Paramaecia can be accelerated by the presence of a certain proportion of chloroform in an otherwise normal medium.

3. Stentor and Paramaecia differ markedly in their division-reaction when subjected to the same substances under similar conditions. In other words, the two organisms are *adjusted* to different conditions.

4. Practically the division-effects which are obtainable by the temporary application of a given concentration of a substance may be attained by the continuous application of a more dilute solution of the same substance.

General Discussion.

My subsequent experiments have given me an insight into the significance of those just described which is different from what the preceding description would seem to imply.

The work described in the following sections shows that it is very difficult to determine the degree of activity of particular factors, even when such factors have been purposely reduced to the smallest number that the vital conditions of the animal permit. In the preceding experiments we have an unknown mixture of both salts and food, dead as well as living, with an excess of one salt. In view of the control experiment, differing only in the absence of the excess contained in the test, any difference in result can be ascribed undoubtedly to that excess. But in view of the further fact, developed in subsequent experiments, that the simultaneous presence of several substances modifies their individual activity, it is difficult to see how any satisfactory insight into the meaning of the above processes can be obtained from the use of mixtures of large and unknown complexity. This consideration shows that there is a limit to the value of the method previously employed, and also suggests a better plan of experimentation.

With the above reservations, I attribute to potassic chloride a degree of specific action not very commonly observed among salts, but probably of more frequent occurrence than our present means of observation indicate. The acceleration due to chloroform is not at all different from that common to nearly all poisons used in appropriately low concentrations. In both the above cases there is a reaction-chain having a stimulating substance at one end of the series and the division-reaction at the other. Except by mere speculation, I am unable to interpolate other elements into the chain.

The action of a temporarily applied substance seems to me to depend much on the greater or less permeability of the cells to the substances applied. Either enough of the permeating substance was retained by the organism to keep up the effect, or the modification at first produced by it was strong enough to affect the activities of the organism after the removal of the reagent. To the subject of permeability in general I shall frequently return in later sections.

V. Observations on Single Salts and Water.

I next planned a series of experiments in which the object was to compare the effects of a number of physiological salts upon the division-reaction when applied singly and in the higher ranges of concentration. The number of salts so experimented with was for several reasons small. First, the time required for this and subsequent work was much more than at first had seemed probable. The precautions taken in making the

preliminary preparation, much of which is described in the section on General Methods and Technique (p. 444), frequently consumed the major part of the time and labor expended. Secondly, the problems suggested by the results with these substances could not be solved by multiplication of similar experiments with other substances. A new line of experimentation was required, and one suggested itself.

Mass-cultures were carried on simultaneously with the special experiments here described, and in most cases furnished the animals for the latter. From these cultures it became more and more evident that different species of Protozoa present peculiar and characteristic adjustments. The observations that gradually impressed this fact upon me are described, but not in their full detail, in the part devoted to the Conditions of Growth in Stentor and the Management of Cultures. This important fact of *specific adjustment* suggests that cumulative experience with a single organism is the necessary preliminary basis for any reliable interpretation of comparative experiments with differently adjusted Protozoa. Accordingly I confined my subsequent experiments almost solely to *Stentor coeruleus*. I regret that limited time and more pressing questions prevented the extension of these experiments to other Protozoa. However, in compensation for this deficiency I have obtained greater certainty for the conclusions drawn, even upon problems of a general nature, by confining my attention to Stentor.

I selected potassic chloride, sodic chloride, calcic chloride, and magnesic chloride as being four fairly representative physiological salts for animal cells in general, as is shown both by ash-content and by feeding experiments (Forster, '73, and others). Since they represent important chemical classes of substances, it was thought that they might facilitate a possible chemical interpretation of physiological action. Furthermore, chlorides alone were selected in order to reduce the number of factors involved and to provide a common factor that would make comparison more practicable. The selection of the chloride, rather than some other radical, though a matter of judgment, was to a certain extent a matter of hazard. It proved to be a fortunate venture. Tests made at various times between the different experiments hereafter described convinced me that the chloride is better adapted to the end in view than any other inorganic radical of the bases used. The chlorine ion is, upon the whole, less injurious and exhibits fewer abrupt gradations in physiological action from salt to salt among the salts here used. This point is always capable of a satisfactory test by selecting different bases with the same acid radical, and *vice versa*. Another organism than Stentor might of

course show a different adjustment. The most difficult preliminary problem in the plan of experimentation was the determination of a common physico-chemical condition for comparison. If solutions of the salts here used be so made as to contain *equal molecular concentrations*, they will still differ in *osmotic concentration* (the latter term used as by Hamburger, :02, p.14). Both of these factors are physically active in any given solution, and biological experiment has shown that they are also distinguishable in their physiological action. To facilitate inference from effect to cause, it seemed advisable to eliminate if possible one of the two factors. There is, in general, a closer correspondence between the intensity of physiological action and the osmotic concentration of a solution than there is between physiological effect and the molecular concentration producing it. Hence the desired elimination of a factor was most easily accomplished by experimenting, in the beginning at least, with solutions of *equal* osmotic concentrations. The molecular concentrations of course *varied*, and differences (or equalities) in the effects obtained could be interpreted accordingly, other conditions being alike. Curves (p. 473), plotted from the mean results so obtained, represent directly the variation in effects which was produced by variation in the molecular concentration of each salt.

Five series of experiments were made, each series representing the simultaneous application of the above selected salts. These series are designated both in the tables which follow and in the curves on p. 473 by the Arabic numerals 1 to 5. Excepting the curves for series 5, the Roman numerals I, II, III, IV, signify potassic chloride, sodic chloride, calcic chloride, magnesic chloride, respectively. The effects of different salts at nearly equal osmotic concentrations would be represented by an additional set of curves (not shown) connecting points numbered with the same Arabic numerals (excepting the 5's, which represent different molecular concentrations of calcic chloride). Between the points designated by the same Arabic numerals, e. g., all the 1's, the secondary curves should be so drawn as to connect these points of equal osmotic pressure in the order of their highest mean results. This order illustrates the variation in mean results when osmotic pressures are equal, better than connection in the order of concentration would. However, either method of connection shows the great lack of parallelism between the curves and the axis of abscissas, and this is the point of importance.

To make such solutions, I took seven tenths as much of a molecular weight of calcic chloride or magnesic chloride as of potassic chloride or sodic chloride. The limits of error on the biological side are so wide

that I abandoned my original intention of making the solutions used strictly isotonic, as measured by the dissociation coefficient, *i*.

Closely fitting solid watch-glasses only partially filled with the liquid medium were used. The test media consisted simply of distilled water containing, accurately, the specified concentration of the salts. The control medium, when used, was a filtered culture liquid from the native culture of the Stentors employed. In presenting the results of experiments the origin of the Stentors is always stated by giving the number of the mass-culture. Nearly all of the animals used in the following experiments came from the same or a few continued cultures. The results are therefore all the more comparable. Customarily I placed ten Stentors in each dish, and prepared either five or ten dishes (in all fifty or a hundred Stentors) of each medium to be tested. All the preparations described under the serial number of a single experiment were made simultaneously and carried on under strictly identical surroundings. Further details of the above procedure have been given in the section on General Methods and Technique (p. 444).

The mean result states the number of individuals to which, on the average, one Stentor increased or diminished in the given time. My general experience in the use of solutions has taught that for my purposes the number of molecular parts in 100,000 is an appropriate measure of their concentration from a physiological point of view.

Series 1. Expt. No. 45. April 8, 1902.

(All Stentors originated from culture No. 20318.)

.01000 m. KCl.

Time	0 hr.	29 hr.
No. of Stentors	100	6?
No. of Divisions	0	0
Mortality		94
Mean Result		0.06

.01000 m. NaCl.

Time	0 hr.	29 hr.
No. of Stentors	100	0
No. of Divisions		0
Mortality		100
Mean Result		0

.00700 m. $CaCl_2$.

Time	0 hr.	29 hr.	4 da.	9 da.
No. of Stentors	100	102	103	105
No. of Divisions		2	3	5
Mortality		0	0	0
Mean Result		1.02		

.00700 m. $MgCl_2$.

Time	0 hr.	29 hr.
No. of Stentors	100	0
No. of Divisions		0
Mortality		100
Mean Result		0

Control.

(Culture medium No. 20318.)

Time	0 hr.	29 hr.
No. of Stentors	100	104
No. of Divisions		44
Mortality		0
Mean Result		1.04

Series 2. Expt. No. 46, Apr. 10, 1902.

(All Stentors originated from culture No. 20318.)

.00500 m. KCl.

Time	0 hr.	28 hr.	47 hr.	5 da.
No. of Stentors	100	88	84	61
No. of Divisions		0	0	
Mortality		12	16	
Mean Result			0.84	

.00500 m. NaCl.

Time	0 hr.	27 hr.	47 hr.
No. of Stentors	100	20	0
No. of Divisions		0	0
Mortality		80	100
Mean Result			0

.00350 m. $CaCl_2$.

Time	0 hr.	29 hr.	46 hr.	5 da.
No. of Stentors	100	91	88	91
No. of Divisions		0	0	3
Mortality		9	12	
Mean Result			0.88	

.00350 m. $MgCl_2$.

Time	0 hr.	27 hr.
No. of Stentors	100	0
No. of Divisions		0
Mortality		100
Mean Result		0

Series 8. Expt. No. 50, Apr. 14, 1902.

(All Stentors originated from culture No. 20318.)

.00300 m. KCl.

Time	0 hr.	24 hr.	48 hr.
No. of Stentors	100	90	91
No. of Divisions	10	0	1
Mortality		10	10
Mean Result			0.91

.00300 m. NaCl.

Time	0 hr.	24 hr.	48 hr.
No. of Stentors	100	80	85
No. of Divisions		0	5
Mortality		20	20
Mean Result		0.85	0.85

.00210 m. $CaCl_2$.

Time	0 hr.	24 hr.	48 hr.
No. of Stentors	100	99	101
No. of Divisions		0	2
Mortality		1	1
Mean Result			1.01

.00210 m. $MgCl_2$.

Time	0 hr.	24 hr.
No. of Stentors	100	2
No. of Divisions		0
Mortality		98
Mean Result		0.02

Series 4. Expt. No. 48, Apr. 12, 1902.

(All Stentors originated from culture No. 20318.)

.00100 m. KCl.

Time	0 hr.	43 hr.	3 da.
No. of Stentors	100	105	109
No. of Divisions		5	9
Mortality		0	0
Mean Result		1.05	

.00100 m. NaCl.

Time	0 hr.	43 hr.	3 da.
No. of Stentors	100	106	117
No. of Divisions		6	17
Mortality		0	0
Mean Result		1.06	

.00070 m. $CaCl_2$.

Time	0 hr.	42 hr.	3 da.
No. of Stentors	100	99	105
No. of Divisions		0	6
Mortality		1	1
Mean Result		0.99	

.00070 m. $MgCl_2$.

Time	0 hr.	42 hr.	3 da.
No. of Stentors	100	80	77
No. of Divisions		0	0
Mortality		20	23
Mean Result		0.80	

It is evident from the preceding experiments that for the three salts, potassic chloride, sodic chloride, magnesic chloride, I have reached the

destructive maximum of concentration. The following experiments were made to determine the same point for calcic chloride:

Series 5. Expt. No. 57, Apr. 17, 1902.

(All Stentors originated from culture No. 20318.)

0.05000 m. $CaCl_2$.

Time	0 hr.	22 hr.
No. of Stentors	100	0
No. of Divisions		0
Mortality		100
Mean Result		0

0.04000 m. $CaCl_2$.

Time	0 hr.	22 hr.
No. of Stentors	100	0
No. of Divisions		0
Mortality		100
Mean Result		0

0.03000 m. $CaCl_2$.

Time	0 hr.	22 hr.
No. of Stentors	100	0
No. of Divisions		0
Mortality		100
Mean Result		0

0.02000 m. $CaCl_2$.

Time	0 hr.	22 hr.	49 hr.
No. of Stentors	100	16	11
No. of Divisions		0	0
Mortality		84	89
Mean Result		0.16	0.11

.01000 m. $CaCl_2$.

Time	0 hr.	22 hr.	49 hr.
No. of Stentors	100	51	49
No. of Divisions		0	0
Mortality		49	51
Mean Result		0.51	0.49

.00700 m. $CaCl_2$.

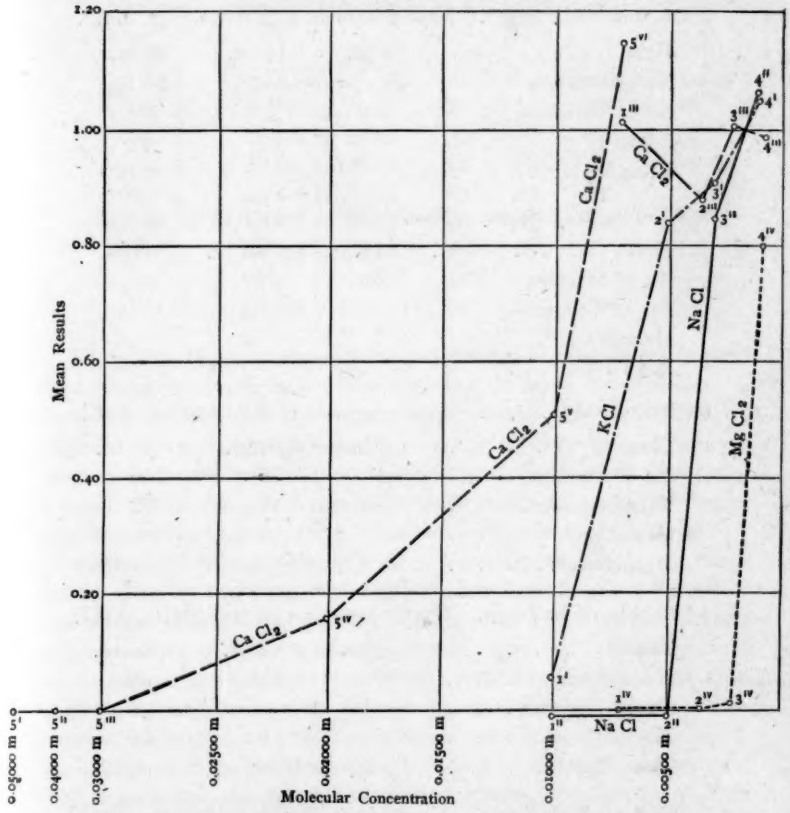
Time	0 hr.	22 hr.	49 hr.
No. of Stentors	100	115	109
No. of Divisions		15	15
Mortality			6
Mean Result		1.15	1.09

Control.

(Culture medium No. 20318.)

Time	0 hr.	26 hr.
No. of Stentors	100	127
No. of Divisions		27
Mortality		0
Mean Result		1.27

The results of the experiments thus far described and plotted in the accompanying curves (p. 473) permit some inference as to the relative magnitude of the *factor of molecular concentration*, and to this subject I shall recur in detail later. To get an independent estimate of the *osmotic factor*, I determined to make some experiments in which this should be the principal factor involved. Some trials were made with cane sugar, but the samples used were probably contaminated and not sufficiently recrystallized. However, the most serious objection to this substance, for my purpose, was its strong tendency to acid fermentation. The presence of acid occurs so soon after the solution is made as to become an important factor in results taken after twenty-four to forty-eight hours or more. After several trials the use of cane sugar was abandoned. Fortunately milk sugar proved to be practically innocuous to Stentor, and it has the advantage over cane sugar that its fermentation is very slow. Tolerably pure samples were purchased and freed from dust and other contaminations by repeated crystallization from hot solution. Parallel with the application of milk sugar I applied to Stentor of the same origin a concentration of 0.00700 m. sodic chloride, sufficient, according to previous experience, to produce a marked effect. I selected sodic chloride for comparison because of its want of any marked specific effect upon division (cf. potassic chloride). The osmotic concentration of a 0.00700 m. sodic chloride solution is about equivalent to that of a 0.01250 m. milk-sugar solution, and this latter is the lowest concentration of milk sugar used in this experiment. For comparison with the salts, the application of a substance (milk sugar) found to have practically no other



For explanation of curves, see pp. 480–492; for discussion, see § 6, pp. 480–492.

influence upon Stentor than that of osmotic pressure is well adapted to show the relative magnitudes of the osmotic and the specific factors.

Expt. No. 55, May 14, 1902.

(All Stentors originate from culture No. 20502.)

0.01250 m. Milk Sugar. Nearly isotonic w'th 0.00750 m. NaCl.

Time	0 hr.	10 hr.	48 hr.
No. of Stentors	50	54	53
No. of Divisions		4	4
Mortality			1
Mean Result			1.06

0.01500 m. Milk Sugar. Nearly isotonic with 0.00900 m. NaCl.

Time	0 hr.	16 hr.	48 hr.
No. of Stentors	50	55	56
No. of Divisions		5	6
Mortality			0
Mean Result			1.12

0.01750 m. Milk Sugar. Nearly isotonic with 0.01050 m. NaCl.

Time	0 hr.	16 hr.	48 hr.	5 da.
No. of Stentors	50	50	54	46
No. of Divisions			4	
Mortality			0	
Mean Result			1.08	

0.02000 m. Milk Sugar. Nearly isotonic with 0.01200 m. NaCl.

Time	0 hr.	16 hr.	48 hr.
No. of Stentors	50	56	59
No. of Divisions		6	9
Mortality		0	0
Mean Result			1.18

0.02500 m. Milk Sugar. Nearly isotonic with 0.01500 m. NaCl.

Time	0 hr.	16 hr.	48 hr.
No. of Stentors	50	51	56
No. of Divisions		1	6
Mortality		0	0
Mean Result			1.12

0.03000 m. Milk Sugar. Nearly isotonic with 0.01800 m. NaCl.

Time	0 hr.	16 hr.	48 hr.	5 da.
No. of Stentors	50	53	56	57
No. of Divisions		3	6	7
Mortality		0	0	0
Mean Result			1.12	

0.00700 m. NaCl. Nearly isotonic with 0.01250 m. Milk Sugar.

Time	0 hr.	3.5 hr.	30 hr.
No. of Stentors	50	5	0
No. of Divisions		0	0
Mortality		45+	50
Mean Result			0

To facilitate comparison this experiment is summarized in the following table:

Milk Sugar.	Isotonicity.	0 hr.	16 hr.	48 hr.	Mean Results.
.01250 m.	.00750 m. NaCl	50	54	53	1.06
.01500 m.	.00900 m. NaCl	50	55	56	1.12
.01750 m.	.01050 m. NaCl	50	50	64	1.08
.02000 m.	.01200 m. NaCl	50	56	59	1.18
.02500 m.	.01500 m. NaCl	50	51	56	1.12
.03000 m.	.01800 m. NaCl	50	53	56	1.12
NaCl. .00700 m.	.01250 m. $C_{12}H_{24}O_{12}$	50	5	0	0

These data on milk sugar do not seem to me to be sufficient to yield any satisfactory conception of *how* the salts act upon the organism. I have worked out only the higher range of the concentration curve, whereas from a physiological point of view it is a lower range of concentrations that represents normal conditions and is therefore of especial interest. From the logical standpoint the investigation might have begun with the action of pure water. Perhaps by this method additional factors could have been introduced into the physico-chemical reaction chain sooner than by beginning with higher concentrations. A few tests of the reaction of Stentor to ordinary distilled water and to various samples of supposedly pure water were suggestive of a new and interesting phase of the general problem treated of in this research. It was evident upon preliminary analysis that the physico-chemical phase would have to be worked out carefully before the biological aspect could be approached. As a measure of the purity of water I adopted its electrical conductivity. This is altogether the most delicate and practicable of the known methods for the end in view. For the measurement of conductivity I adopted the Nernst apparatus (Nernst, '94, and Maltby, '95). For details of the construction and use of the apparatus, and for the preparation of water for these tests, I refer to the original articles and to the part of this paper dealing with General Methods and Technique (p. 444).

I determined that the average conductivity of the ordinary distilled water which I used was about $\kappa = 6.8 \times 10^{-6}$, and I also prepared a quantity of water whose conductivity was $\kappa = 0.9$ to 1.0×10^{-6}. An estimation of the degree of purity these numbers represent and of the probable nature and importance of the contaminations present are stated or referred to in Kohlrausch u. Holborn ('98, pp. 111 ff). Suffice it to say here that the purest water I used as a reagent for Stentor

($\kappa = 1 \times 10^{-6}$, or $1- \times 10^{-6}$) was very nearly as pure as one can produce when working in contact with air. This statement assumes of course that the containing vessel in which the experiment is performed is not a source of contamination. Wherever this possibility was a factor of importance I have taken pains to prove the absence of contamination by the objective method of a conductivity determination. In some cases, depending on the end in view, it suffices to know the degree of contamination or its rate, or the fact that the contamination is so small in a given time as to defy detection by even so delicate a method as that here used.

What, then, are the phenomena of the reaction of Stentor to pure water? I will first describe this experiment as it presents itself to simple direct observation. The distilled water used, if freshly prepared, should be aerated by repeated pouring from one vessel into another in a thin stream of some height. I applied both the ordinary distilled water which I was in the habit of using and such as had a conductivity of $\kappa = 1- \times 10^{-6}$. Great care is necessary to avoid the introduction of contamination with the organisms, as it is of course impossible to handle them except as they are contained in liquid. The method of multiple transference, described on pp. 445–446, meets the requirements and permits an objective (conductivity) test of its efficiency in any given case. Quadruple transfers were made in this test. The solubility of the glass used proved upon examination to be a negligible quantity in this experiment. The animals show at first no noticeable signs of discomfort. If undisturbed they gradually come to rest in an expanded condition, as they do in any harmless medium. But after about one and a half to two hours in the water of $\kappa = 7 \times 10^{-6}$, or after about three fourths of an hour to an hour in the water of $\kappa = 1- \times 10^{-6}$, the first evidence of disintegration appears in the form of irregularities of the surface. The margin of the optical section of the Stentor looks roughened instead of smooth, as it normally is. Within a short time afterwards there remains only a mass of disintegrating material. The two samples of water yield the same qualitative phenomena, but the onset of disintegration occurs sooner in the pure water. The length of time that the animals resist pure water depends of course upon their physiological condition and is therefore variable. A sample of water causing disintegration within a few minutes (complete destruction within fifteen minutes, as I once observed) justifies the suspicion of its containing solid or gaseous impurities that are foreign to properly distilled water. Experience with personally prepared and tested waters will amply prove this statement. The treatment of Stentor with more or less pure distilled water was frequently

employed in the course of this investigation, and always with essentially the result above described.

While it is possible to make an inference from the experiments with salts and with water, as to the method of destruction by distilled water, much more satisfactory objective evidence is furnished by the next experiment. This consisted essentially in the measurement of the changes in electrical conductivity exhibited by very pure water in which Stentors were contained. It is true that my original object in making this experiment was to obtain a quantitative expression for the respiratory activity of these small organisms. The measurement of CO_2 excretion seemed *a priori* feasible. The result in the sense in which the question was put was negative, but the facts developed are of material importance to a general explanation of the action of water and salts as observed in my experiments. To the subject of respiration I shall return in the section on permeability.

For measuring the conductivity of the medium I employed the Nernst apparatus previously alluded to. Important sources of error that threaten to vitiate the results of so delicate an experiment need to be carefully eliminated. One of these is the change in conductivity due to change of temperature, and another is the introduction of contamination into the medium from the solution of portions of the apparatus itself. As is well known, increase in temperature increases the absolute conductivity, κ, of liquids, and *vice versa*. A favoring circumstance in the present experiment is that the temperature coefficient of pure water is comparatively small, but constant values are not known (Kohlrausch u. Holborn, '98, p. 115), and if any temperature change occurs during the experiment its corresponding change in conductivity must be determined for the case in hand. This latter I have done when necessary.

The portion of the apparatus requiring the most scrupulous control of conditions is the test-cell in which the medium with the Stentors is placed. I have found by experience that good chemical glassware, such as is used for beakers, frequently furnishes, especially after usage or preliminary treatment (Kohlrausch u. Holborn, '98, p. 113 ff), a glass vessel of such resistance to solution as to give no electrically measurable quantity of solute within the length of time occupied by my experiment. The thimble-sized beaker here used, capable of containing about 5 cc., was of this nature. Because of the high resistance of the medium (pure water) I was able to use *unplatinized* electrodes without experiencing any difficulty from polarization in obtaining a good minimum in the telephone. Aside from this, freedom from polarization is one of the

important advantages of Nernst's apparatus. The circumstance of being able to use unplatinized electrodes relieved me of what would otherwise have been an unavoidable source of contamination. The electrodes were fixed at adjustable distances apart upon a frame of hard rubber, which, when set over the thimble beaker, suspended the electrodes in the contained water. Between them, but a little to one side, was suspended a mercurial thermometer with bulb immersed. This arrangement of test-cell is not recommended as the best that could be constructed to serve the required purpose. It is described here principally to give information of the conditions under which the experiment was performed. It results from the construction that glass (of beaker and thermometer), platinum, and air are the only objects in contact with the contained liquid. If necessary the bulb of the thermometer and the inside of the beaker could be coated with paraffine. To test the efficiency of the conditions, I made the following control experiment. The test-cell was set up with a content of 3 cc. of very pure water. Practically simultaneous readings of time, temperature, and the length (L) of the measuring column of resistance-liquid were made and recorded. The test-cell was connected in parallel with the measuring tube; it must be remembered that in this mode of connection the length (L) increases with the conductivity of the liquid in the test-cell and *vice versa*. The numerical value of the conductivity is not important to my argument. With the mirror scale and the magnifier with cross-threads that were in use when this experiment was made it was possible to read one twentieth of a scale-division, one tenth of a scale-division being a change in length of significance, the scale-division being 1.2 mm. The position of the electrode was read directly. The dial attachment (Nernst, '94, and Maltby, '95) for very much smaller readings was not used in this experiment.

Under the control conditions above described the following record was secured.

Time,	8.30	8.37	8.45	8.55	9.05	9.15
Temp.,	21°.00	20°.85	20°.75	20°.70	20°.70	20°.75
L.,	10.5	10.5	10.5	10.5	10.5	10.5

Time,	9.25	9.30	Variation = +60 minutes.
Temp.,	20°.80	20°.90	Variation = −0°.1 C.
L.,	10.5	10.5	Variation = 0 divisions.

The result shows satisfactorily that the apparatus itself (that is, the test-cell) is not a source of contamination to the contained medium, when in use for a period of one hour.

I now placed in the same test-cell 3 cc. of very pure water containing ten Stentors introduced by the process of multiple transference, and made a series of observations corresponding to the above with the following result:

Time,	4.07	4.15	4.20	4.27	4.31	4.38	4.47
Temp.,	23°.30	23°.30	23°.35	23°.35	23°.30	23°.15	23°.00
L.,	10.6	10.6	10.6	10.6+	10.6+	10.65 (.7?)	10.65

Time,	4.54	5.07	Variations = +60 minutes.
Temp.,	23°.00	23°.00	Variations = −0°.30 C.
L.,	10.7	10.7	Variations = 0.1 divisions.

Since there was a decrease in temperature, a correction of L for this would result in a larger value than 0.1 scale-division. Although it is not important to my purpose to establish more than an appreciable value for L, a rough trial was made immediately with the same materials, but with a temperature change of 2° C. This was equivalent to a change of 0.5 scale-division. This correction applied would give a value to L of about 0.17 scale-division.

I will record here one more similar experiment. The same thimble-beaker with paraffined thermometer bulb and 3 cc. of pure water only, gave the following readings for the control experiment:

Time,	3.02	3.10	3.17	3.25	3.32	Variations = +30 min.
Temp.,	21°.75	21°.65	21°.50	21°.50	21°.50	Variations = −0°.25 C.
L.,	10.5	10.5	10.5	10.5	10.5	Variations = 0 div.

With ten Stentors added I obtained the following results:

Time,	3.35	3.50	3.56	4.05	4.18	4.28
Temp.,	21°.50	21°.50	21°.50	21°.50	21°.50	21°.50
L.,	10.5	10.5	10.5	10.5	10.5	10.6?

Time,	4.35	Variations = +60 mimutes.
Temp.,	21°.50	Variations = ±0° C.
L.,	10.6	Variations = +0.1 divisions.

Experiments like the above two were often performed, but not always with the care here exercised. *The results uniformly showed an increase in the conductivity of the water containing the Stentors.* I have however learned to regard the above values of L as not being proportioned to the number of Stentors employed.

One further feature of these observations I desire to emphasize here. The increase in value of L above observed did not continue indefinitely, but was only initial, at least in the above intensity. To the meaning of this fact I shall recur (p. 505).

Sufficient data have now been secured at different points on the concentration curve to furnish at least the basis for a general interpretation, and I therefore defer the description of further experiments upon combined salts until after the following general discussion.

VI. Discussion of Single Salts and Water.

I desire to interpret the preceding experiments from two initially different points of view. From one standpoint I shall attempt a physico-chemical explanation as far as the facts warrant; from the other I shall seek to explain the same and additional facts as the expression of specific adjustments in the metabolism of Stentor. The two views are certainly complementary to each other. Whether or not they can be merged into one, I prefer to leave to the reader's opinion upon a fundamental question in biology.

The results of the two experiments last described, together with others that are similar, show that when Stentors are transferred from their ordinary mass-culture medium to very pure distilled water, an increase in the conductivity of the water takes place during at least the initial hour or two. What is the source of this variation? The control shows that it cannot be from any part of the test-cell. It might be urged that the observed increased conductivity was due to the unavoidable introduction of a small amount of the culture medium with the Stentors, notwithstanding the multiple transference of the animals; for the concentration in the immediate vicinity of a solid object, whether animate or inanimate, would be much greater than in the surrounding medium, generally, and furthermore the ciliate covering of Stentor would certainly increase considerably the danger of the retention by the animal of more highly concentrated solution immediately around it at each transfer. But that this cannot be the true explanation of increased conductivity is evident from the fact that this increased conductivity does not manifest itself promptly, but only after the lapse of some time. These facts leave us room for no other explanation as to the cause of increased conductivity than the metabolism of the organisms themselves. Reflection upon the change in the conditions presented by the Stentors when transferred shows, furthermore, that this conclusion is entirely reasonable.

There is abundant evidence that the culture media contain salts. The

later, artificial cultures that furnished material for the present work were raised in favorable salt media, as described in other sections. That the earlier, natural cultures brought into the laboratory from the ponds also contained salts in considerable quantities is scarcely open to doubt. Decaying organic matter and earth in contact with water are abundant sources of salts in solution. Furthermore, evaporation of a few cubic centimeters of culture medium always left a perceptible residue. Conductivity measurements of the natural media, determined by comparison with solutions of calcic chloride, usually gave a value equivalent to that of a 0.00100 m. to 0.00200 m. calcic chloride solution (see p. 508). From whatever culture the organisms for this experiment came, it is certain that their original medium contained a greater concentration of salts than the very pure distilled water to which they were transferred.

The presence of salts in the medium does not necessarily imply that the cell living in it contains the same salts. The discovery of the relation between the salts in the medium and those contained in the free-living cell itself, being the object of the present investigation, this relation is not to be assumed. For example, in Chaetomorpha and Spirogyra, according to Jansen (cited by Schäfer, '98, p. 277), we have illustrations of a great difference between the concentration of the salt within the cells and that in the surrounding media. The two plants have about the same internal osmotic pressure, although the former is a marine, the latter a fresh-water form, Spirogyra thus having a greater and Chaetomorpha a less pressure than their respective media. The permeability of the organism, which probably governs such conditions, will receive some attention for Stentor in a later section. Here will be made only the very probable assumption that the Stentor cell does contain a certain quantity of salts. That Stentor contains physiological salts in certain proportions may be inferred from the results of chemical analysis in the case of all organisms obtainable in sufficient quantities to permit such analysis, and from the results of all experiments on nutrition made with reference to salts (e. g., Forster, '73, Herbst, '92–98, and others). The experiments upon adjustment given in a later section of this paper also lend support to the proposition that Stentor contains some of all the salts found necessary for a medium in which its metabolic processes may normally take place. What these salts are individually in the case of Stentor is not of importance at this stage of the discussion; but I may point out that the salts within the cell probably originate from the medium, either directly, by osmotic absorption and by ingestion, or indirectly, being contained in other organisms used as food, which organisms origi-

nally obtained their salts from the medium. It is assumed here that although some of the salts contained in the cell may possibly be in a state of chemical combination with proteid, yet a considerable proportion of them exist in a condition to produce osmotic phenomena. They are either free and in simple solution (in vacuoles, Bütschli), or at most in such unstable combination, physical or chemical, as to be easily broken down into more numerous portions, thereby becoming osmotically more active.

When, therefore, Stentors are transferred from a normal medium to very pure distilled water, there results the osmotic relation of a salt pressure within the Stentor cell that is higher than that existing in the distilled water externally adjacent to it. What process ensues under these conditions depends, at least in large part, upon the degree of permeability of the protoplasm, including the walls of the protoplasmic alveoli (Bütschli) as well as the cell-walls. If the cell-wall of Stentor had been wholly impermeable to salts, much water would have been absorbed (assuming of course free permeability for water), no increase of salts would have taken place in the distilled water, and the animals would have died of swelling; but neither of these phenomena was exhibited. *On the contrary, the evidence that there is an increased salt-content in the distilled water demonstrates the greater or less permeability of Stentor to physiological salts* when the animal is surrounded by distilled water. But I will note in passing that I do not conceive the permeability of Stentor when in very pure distilled water to be the same as when the cell is surrounded by the normal conditions of partial pressures of salts. I have been led to this opinion by the results with milk sugar, as I shall presently explain. We are now able to assign *loss of salts as the chief factor in the destruction produced by distilled water*, possibly brought about, as implied above, by an abnormal condition of the cell-wall induced by contact with water free from salts. The more permeable the cell-wall, the more rapid would be the loss of salts, and the less, consequently, would be the absorption of water. Careful observation failed to show any evidence of swelling due to an increased water-content, which, however, probably took place to some extent. Evidently the loss of a portion of its salts has much more serious consequences for the animal and its metabolism than some increase in its water-content. I must here emphasize, for the purpose of a subsequent comparison (p. 500), the fact that *all* the permeating salts share in this process of withdrawal.

The phenomenon of shrivelling, observable when molecular or hypermolecular concentrations of either physiological salts, or of practically

indifferent substances (sugars, e. g.), are applied to Stentor, permits us to make at least the following inference. Shrivelling is due to loss of water from the cell to the solution outside it, and this movement of water proves that *the Stentor under these osmotic conditions is permeable to water*, a fact which it might perhaps have been permissible to assume rather than demonstrate. But whether loss of those salts which have unbalanced partial pressures was also a factor in the process of destruction by concentrated solutions, is a more difficult question to decide from the evidence. The conditions here are so closely related to those in the milk-sugar experiments that I will consider them in connection with the latter.

We have now considered the conditions that prevail at opposite ends of the concentration curve, and shall next proceed from both regions towards the isotonic or normal region. That portion of the curve which extends from distilled water up to those osmotic pressures which are approximately isotonic with the natural culture media from which the animals for experiment originate, I shall term the *hypisotonic* range. The conditions here require separate description, which I will therefore defer till I have given (in the next section) the experiments on the lower concentrations of single and combined salts.

There will remain, then, the question of the effect and the mode of action of those concentrations which lie in the *hyperisotonic* range (above about 200 molecular parts of calcic chloride in 100,000) of the curve. A comparison of the mean results presented on the one hand by the curves (p. 400) for the four salts, and on the other hand by the milk-sugar experiments, shows that we have to distinguish between two well-marked classes of effects. *The application of milk sugar in hyperisotonic concentration was practically harmless*, whereas like concentrations of the four salts were destructive. Mean results of unity, or something above unity in the former case, and of zero in the latter, show the strong contrast between the two kinds of bodies. The curves (p. 473), taken in connection with the sodic chloride control, give sufficient indication of the general reaction of Stentor to these concentrations to show what would have been the effects obtained from a simultaneous trial of each of the four salts in parallel with each test with milk sugar. Mean results of zero would have been their uniform outcome.

In what way shall we picture to ourselves the processes that bring about these contrasted effects? If Stentors be removed from their native mass-culture liquid to a solution of a single salt of higher concentration, what processes follow?

Of physico-chemical phenomena we have, assuming the permeability of the cell to water, the loss of water from the cell to the solution outside of it, because its osmotic concentration within the cell is in excess of that outside. This process will take place both in the application of the four salts and of milk sugar, but with a very probable difference in degree, depending upon a difference in permeability of the cell to the two kinds of substances. Milk sugar belongs to a class of substances, the sugars in general, found to have very low, if any, power of permeation. Upon the other hand the physiological salts, and especially ionic constituents of them, have been found by Hamburger and others to have more or less power to permeate cells. We now have a better knowledge of the permeability of the mammalian blood-corpuscle than of any other free animal cell. Although the subject of its permeability with reference to particular substances, especially electro-negative radicals (ions) of salts, has been much in dispute, certain results are now established with considerable certainty. These are well summarized in tabular form by Hamburger (:**02**, p. 260). As the results — thus far much too meagre in proportion to the importance of the subject — upon different cells accumulate, it becomes evident that among different kinds of cells much similarity exists in regard to the permeating power of such classes of substances as sugars, alkali salts, and possibly alkali earths. This makes the conclusions above stated regarding the permeating power of milk sugar and the four salts, as well as the quantitative difference between the two, in the case of Stentor extremely probable. The experimental evidence which I have thus far worked out, as described in the section on Permeability (p. 503), is very limited, but such as it is it coincides with the results found in other cells.

Returning from this necessary digression to the case of Stentor in hyperisotonic media, we would have a higher osmotic concentration of milk sugar and of single salts, respectively, outside the cells than inside them. Granted non-permeability in the former case, and a certain degree of permeability in the latter, more water would be abstracted from the cells by milk sugar then by the salts. For the inward movement of the salts or their ions would tend to establish as high osmotic concentration of these salts within the cell as existed outside of it. This process of equilibration would be absent from the cells in milk sugar. We may now answer with much probability of correctness the following important question. In the total destructive effect produced by the application of hyperisotonic concentrations of these four salts respectively, is the abstraction of water, i. e., the simple osmotic factor, of determining

influence? It is not. For while the abstraction of water was greater in milk sugar than in the salts, the mean results show that milk sugar was, in comparison with the salts, practically harmless. It is true that abstraction of water by sufficiently high concentration of sugar, or of inactive salt, if such there be, may be at least a prominent factor in the destruction of the cell. But at present we are describing lower, though still hyperisotonic, concentrations, where there is the possibility that both osmosis and a metabolic activity of the same substance are present. The demonstration of these two modes of action, and especially their separate estimation, is the first important step toward a physico-chemical analysis of the action of physiological salts upon cells. *We have now carried the analysis far enough to be able to exclude abstraction of water as the determining factor in the destruction produced by these salts at the higher ends of the curves.* Where, then, shall we look for the efficient factor?

I now return to the process of equilibration which takes place when the cell is more or less permeable to the salt. With the outward movement of water there is also, because of unequal salt pressure already described, a concurrent inward movement of the *single* salt applied (or of its ions). There results an introduction into the cell of an excess of that salt in proportion to other salts present in normal amount. We have to deal, then, with the physiological effect of an excess of one salt within the cell, a normal proportion of which would not interfere, in the case of physiological salts, with its usual metabolism. *The ruin of the cells in the given hyperisotonic solutions was due to an excessive proportion of a single salt.*

In the next section I shall present experimental evidence that there is an increased division-reaction with Stentor under the influence of several salts together, as compared with that under the action of one, and especially of the adjustment of Stentor to *limited proportions* of different salts.

From the experiments with distilled water it might be suggested here that an objective determination should be made — either by electrical conductivity or by quantitative chemical estimation — to prove that water has increased or that salt has proportionately diminished in the external medium. This was not done because the above described conditions of concentration are made sufficiently certain by the ascertained osmotic concentration of the native mass-culture medium of the Stentors and the purposely prepared higher concentration of the reagents used. Granted a certain degree of permeability, of which the experiments themselves furnish evidence, the above described processes tending to equilibration of pressure follow as a physical necessity. Furthermore

there would be required a correction (as explained in the section on Permeability) for change of volume of the Stentors used, which was, from the nature of the case, not required in the experiment with distilled water.

That the destructive action of the excess of a single salt was not osmotic in its nature, is evident from the absence of injury under the influence of milk sugar in a concentration exerting equal or greater osmotic pressure. Mechanical pressure and movement of water, characteristic osmotic processes, resulted in no harm to the cells. Simple osmotic action, that is *redistribution* of water and salts, as it would occur in a physical experiment, is not sufficient to account for the result, even though the curves may be subdivided into osmotic regions. In fact, the experiments upon Stentor, taken as a whole, permit the conclusion that osmosis, though a factor necessarily present, is secondary in importance to the *specific* activity of physiological salts. The modification of membranes included in that class of phenomena denominated by Ostwald "mechanical affinity" (Schäfer, '98, p. 275), and the distinctively chemical processes included in metabolism, exert a controlling influence superior to that of simple osmotic distribution of salts and water. An important problem suggests itself here, namely, the determination of the specific activities of the salts with reference both to permeability and to their individual metabolic functions.

It might be supposed that the physiological salts, not being active substances chemically, owe most of their physiological effects to their osmotic action. It is highly probable that the maintenance of a certain mechanical pressure in the medium surrounding a cell is at least *one* of the important functions of physiological salts. But the magnitude of this factor as compared to the chemical, metabolic activity of these salts is strikingly shown by the secondary curves on page 473. As explained on page 466, these are to be so drawn as to connect those concentrations of the different salts which represent approximately equal osmotic pressures. If the salts had produced mean results in proportion to their osmotic pressures these secondary curves would have been parallel to the axis of abscissas. But at equal pressures the mean results have such values as to produce curves, making nearly the largest angle possible with the axis of abscissas. These facts, as represented by the curves, justify the conclusion that *the physiological salts have a distinct chemical, that is, specific activity in addition to their osmotic function.*

We have now attempted a separate estimation of the relative shares, for Stentor, of the osmotic factor and of the qualitative chemical factors,

both of which are exerted by the same substance at a given concentration. Because the latter factor depends upon the nature of the substance whose native properties come to act physiologically *when that substance forms an essential, that is, a constitutive proportion of the living organism*, and because I shall need a well-defined term to express this relation, I shall denominate it the factor of *constitutive proportion* or, here, simply the constitutive factor. The idea of proportion will have importance in the next section.

Substances which act by virtue of being in the organism form a general series of some range with respect to the constitutive and osmotic factors. If the substance is actively toxic the osmotic factor becomes practically *nil*, the constitutive factor accounting for nearly the whole result. A modification of the permeability of membranes through alteration of constitution, which may possibly be important in toxic action, I reckon of course with the constitutive factor. At the opposite end of the general series there is a class of substances, here called the physiological salts, which were once considered to be practically inert (ash) constituents of the organism. But investigations beginning with Liebig have shown the necessity of a different interpretation. Their importance as osmotically active substances is now demonstrated. But their constitutive, metabolic activity, although evidently present, is practically unknown, so far as regards its mode of action. To the question, what is the chemical activity in the case of each salt, physiology is at present able to give but very uncertain answer. Suggestions as to what these activities may consist in are to be found in Rywosh (: 00).

We are now prepared to consider some peculiar conditions in our experiments, made evident by analysis. When the animals were contained in a medium of distilled water, there existed within each the several partial pressures of their physiological salts. On the outside of the organism respective partial pressures to balance the former were absent. Salts were withdrawn and the animals died in consequence. Upon the other hand, when Stentors were placed in a hyperisotonic medium of pure milk sugar exactly the same unbalanced partial pressures existed within the cell, yet they did not die for the want of salts. In fact the animals suffered no appreciable harm from the milk sugar medium. The same unbalanced partial pressures existed when a single hyperisotonic salt was applied, excepting for the single salt in question. Death resulted *mainly* from a penetrating excess of the salt applied. First, how shall we explain the contrasting effects above referred to between milk sugar and distilled water? Evidently some other condi-

tion than simply the unbalanced partial pressures of the salts comes into play. It is well known that in experiments on osmosis a membrane may during the course of the experiment alter its constitution in consequence of having, for example, a greater affinity for the solvent than for the solute. Thus a copper ferrocyanide membrane (Schäfer, '98, Vol. I, p. 275) absorbs water. Some such process as this I conceive to take place with Stentor in distilled water. Absorption of water by the cell-wall or the protoplasmic meshwork might very probably result in a permeability for the contained salts much greater in degree than its normal permeability. Furthermore we do not know what important changes in metabolic processes the presence of more water than normal might entail. The processes of molecular condensation, perhaps indicated by greater or less relative dryness of protoplasm, give indications of being of fundamental importance in the anabolism and katabolism of living organic matter. Whatever the process may be, the presence of an extremely high concentration of water is its efficient condition, and it is this same condition that is absent in the hyperisotonic application of milk sugar and of salts also. If, then, the protoplasmic *membrane* swelled, and so increased its permeability to an abnormal degree in distilled water, such swelling would be absent in hyperisotonic media of milk sugar and physiological salts. The permeability of the cell could be less than normal, especially toward the *outward* movement of its physiologically necessary salts. This condition by no means necessitates the preclusion of the *entrance* of a single salt existing externally under high pressure. Some membranes, for example the shell membrane of the hen's egg and the skin of the frog (Schäfer, '98, Vol. I, p. 280), are known to be permeable to a particular substance in one direction only. The outward movement of salts with unbalanced partial pressures into distilled water *is due to the alteration of the permeability of the membrane by contact with water free from salts.* Milk sugar, on the contrary, does not alter the normal permeability, which presumably does not permit the escape of salts physiologically necessary to the life of the cell. It seems quite probable that abnormal alteration of the important membrane that mediates nearly all interchange between the surrounding liquid and the cell contents, is a frequent, perhaps constant, factor in the action of salts and other agents upon free-living cells. The *physiological condition* of the membrane may embrace such a variety of possibilities that physico-chemical analysis cannot proceed far without the determination of this factor. This is a difficult undertaking, that must be made in each particular case.

The above explanation may be considered only as a possibility, and as showing that there is no necessary inconsistency in the previous explanation of the results with distilled water on the one hand, and on the other, single salts at higher concentration and milk sugar. These considerations further emphasize the importance of permeability in some fundamental physiological problems.

We pass finally to the middle, or approximately isotonic, region of the curves. As these concentrations are approached from either direction it is observable that the values of the mean results tend upward toward unity, or even to a point above that. It is true that the curves given lack a series of values between those afforded by distilled water and by the isotonic region. Data in this hypisotonic range are given in the next following section. Yet it is plain that the addition of even a *single* physiological salt to distilled water produces a rise in the curve that may cause it to reach unity in approximately the isotonic region, as is here actually shown, or possibly even sooner. The corresponding conditions have already been considered to some extent in describing the osmotic relations in normal or native culture media. Upon the previously applied principles of permeability, partial pressures, and the physiological conditions of the cell-wall in the particular experiment, it is not difficult to construct a picture of what probably occurs. It is not necessary to restate these details, but I desire to add the following considerations. The metabolism of the cell will cause the partial pressures of the physiological salts inside the cell to undergo continual and probably unequal fluctuations for the different salts. In other words, anabolic and katabolic changes will result in a continually varying number of salt molecules, and a consequent equilibration of partial pressures, which is, in all probability, unequal for the different individual salts. A slight change in the composition of the external medium would also entail a rearrangement of osmotic pressures. Complete osmotic equilibrium, that is, complete isotonicity, probably never exists at any time during the life of the animal. These continual changes are part of the normal, vital metabolism of the cell. They show the deep significance to the organism of what seems at first to be merely the fulfilment of the necessities of physical law.

The conditions cannot, of course, be entirely normal as long as we apply externally only a single salt. But with even a single salt the approach towards normal conditions becomes evident by a rise in the curve under the following conditions. As the concentration increases from the hypisotonic towards the isotonic region, or as the concentration

diminishes from the hyperisotonic towards the isotonic region, the two limbs of the curve show by their rise an approach to normal conditions. In other words, *all concentration curves of a single physiological salt have the characteristic median elevation with depression towards either extreme.*

We are compelled to admit that the physico-chemical phenomena involved in the interaction of cells and media, while yielding some results in the attempt at analysis, are still very obscure. However, if we begin analysis with the larger and more evident complexes of phenomena (in Roux's sense), we can treat them from another standpoint, that of adjustment. But the description of the phenomena can never be complete without an account of the physico-chemical processes, which are, to say the least, one form of the expression of the life of the organism.

The Adjustments of Stentor.

As my experiments in the treatment of Stentor with salts increased in number, certain apparent inconsistencies, as well as certain constant relations, attracted my attention. There is insufficient detail in the data thus far obtained to explain these conditions by physico-chemical methods. This fact led me, not to the abandonment of physico-chemical explanation, but to consider the phenomena from another point of view also. This has to do with the adjustment of the animal to different conditions.

Before drawing conclusions upon adjustment from the preceding data and the curves partially representing them, it is important to estimate their range of applicability. Are the mean results above recorded, fixed values? I do not maintain that in their absolute, numerical value they are even approximately invariable. Occasionally repetitions with solutions of previously used concentrations gave results surprisingly close to the first ones; but in other cases there was much deviation. Further observation showed this *variation in results corresponded to the conditions of the cultures* from which the animals originated. As I was engaged throughout this research in the management of cultures as my source of material, I had favorable opportunities to become familiar with newly established, with flourishing, and with declining cultures. Experiment convinced me of the great importance of knowing the origin of the Stentors used. Results vary in their numerical value with the vitality, i. e. the physiological condition, of the animals. A Stentor culture as it passes through its successive stages offers excellent examples of this variation. In general I selected as the source of my material only healthy cultures. Perhaps the best criterion of this condition was the longevity of the culture. It is to be especially observed that the curves

of the four salts experimented with give results obtained with animals mostly from one culture, No. 20318, and that these experiments were made within a short period of time. These Stentors originated from a good culture under conditions as nearly representing a physiological norm as can well be obtained. The particular results are therefore thoroughly comparable among themselves. But comparison within the limits of what is practically a single experiment does not show the extent of their applicability. When a certain relation between the effects of given substances has been satisfactorily established within the limits of an experiment, that relation can be transferred logically to other experiments and will be found to be applicable; but it may be pitched upon a relatively higher or lower scale *according to the metabolic condition of the animals.* In other words, nutritional variations within certain limits do not nullify fundamental adjustments of the organism, as one might be led to suppose by a comparison of numerical values obtained at different times. Protozoa are not more capricious in reaction than other groups, their reputation for this resting upon a failure to investigate conditions. There exists for each species a normal physiological condition which the experimenter must learn to recognize. There also exist within the cell, as proved by characteristic reactions, normal substance-relations that are identifiable in series of experiments even when these are conducted at different pitches of metabolic activity. For the detection of these, absolute numerical values are no guide. But the relation of values to each other, and a common standard of comparison, enable us to compare experiments differing widely in numerical results. The possibility of doing this is an important consideration when experiments necessarily extend over a long period of time. Concrete illustrations of this method will be given in this and the following sections.

We may now put the question, What adjustments, that is, what correlations of conditions, in Stentor are shown by the preceding data? Let us begin with the curves (p. 473). An unmistakable feature is their serial arrangement. This is shown by the fact that the rise in the value of the mean result above the zero point occurs serially in the order of diminishing concentration as follows: calcic chloride, potassic chloride, sodic chloride, magnesic chloride. Practically the same order holds for the opposite ends of the curves (or the curves produced), for the points at which the mean results reach the value of unity, as is graphically represented by their intersections with the axis of abscissas. More or less parallelism is evident in the curves, and no intersection with each other occurs on the paths of the curves between the zero points and the axis

of abscissas. Quite other conditions than the above, carrying with them a different significance, are conceivable. The concentration at which a substance *begins* to be favorable for the physiological processes of the animal is shown by the point at which the upward curvature occurs. Since these upward curvatures, and even the paths of the curves as a whole, stand in the serial order above mentioned, *Stentor must be best adjusted to calcic chloride, next to potassic chloride, then to sodic chloride, and least to magnesic chloride.* It is not maintained that in another series of experiments the curvature would occur at the same absolute points of concentration, or that the mean results would reach unity at the same points; but it *is* maintained that, if comparative experiments with corresponding concentrations of two or more of the four salts be made upon Stentors having the same physiological condition, although the results obtained might be numerically different, they would continue to stand in the same *relative* order, the results most favorable to the animal being those for calcic chloride. In other words the curves are valid, as I have previously explained, only *relatively*, not absolutely. Further evidence that calcic chloride favors the life processes of Stentors more than the other salts do can be found in the next following section.

In the case of milk sugar we have a different form of adjustment. The physiological salts being natural constituents of the organisms, adjustment to them may be distinguished from that to milk sugar, which is probably an artificial, though certainly an *indifferent*, substance for Stentor.

The significance of adjustments for the internal organization of the cell will be discussed in the following section.

VII. Observations on Single and Combined Salts.

The experiments of the present section were made with a twofold aim. First, it was desired to complete the physico-chemical description of the action of salts in the hypisotonic range of concentration, the aim being here theoretical; secondly, to determine the *normal adjustments* of Stentor to physiological salts. The purpose was to find such salts and such combined *proportions* of these as seemed most favorable to the life-processes of the animal. From this would result the determination of, first, the *qualitative range of adjustment*, that is, the number and kinds of substances to which the animal reacts normally; secondly, the *quantitative range of concentration* that was normal for the animal in the case of each salt. The two series of facts constitute the ground for the theory of

constitutive proportion referred to in the preceding section. A complete knowledge of an animal's relation to substances, even if only to a class, for example to physiological salts, would require more experiments than I have been able to make. But I have extended my studies far enough to illustrate this method of attacking some of the problems of the internal chemical conditions of a free-living cell.

Polished watch-glasses, closed by piling them on top of one another, were used to contain the salt media and the Stentors. It was customary to make the test for each substance with fifty animals, ten each in five different watch-glasses. In the following records, unless a statement to the contrary is made, there were ten animals to a watch-glass. All the Stentors for a given experiment came from the same culture and they were therefore subject to like conditions. But differently numbered experiments frequently included animals of different origin. Their comparability has been previously discussed. The number of Stentors surviving after the expiration of stated intervals of time are written in their respective columns.

Expt. No. 44, April 2, 1902.

The difficulty in finding a uniformly successful method of raising Stentors in mass-cultures finally led me to give special attention to the salt-content of the media so used. Many such cultures were carried on parallel to the following experiments and with media whose composition was based upon them. From among the salt solutions in common use for the water-culture of plants, I selected Pfeffer's medium (Pfeffer : 00, p. 240), made as follows. Of calcic nitrate 4 grm.; of potassic nitrate, magnesic sulphate, acid phosphate of potassium, each 1 grm.; of potassic chloride 0.5 grm.; all dissolved in 7 litres of distilled water. A preliminary trial showed that much dilution was necessary for use with Stentor. The above medium was diluted to seven times its original volume. It is not necessary for my purpose to determine what may be the chemical condition of the above salts after their interaction in dilute solution. Stentors placed in this medium gave the following results:

One Seventh Pfeffer's Medium.

Time	0 hr.	15 hr.	46 hr.	10 da.	15 da.
No. of Stentors .	50	55	96	99	76

The medium contained no food in the ordinary sense of the term and the numerical results are another illustration of the propriety, under this

circumstance, of taking results for comparative purposes after an approximately forty-eight-hour interval.

Evidently this is a medium to which Stentors are well adjusted. But the addition of salt-containing food, for example hay or leaf extract, gave a mass-culture that was a failure. Possibly such an addition brought about a disturbance of the salt balance, the existence of which will be established by experiments described further on in this section. Inspection of the radicals represented by the salts of this medium shows a duplication between different salts that will render it unnecessarily difficult to determine their separate shares of activity by the method of excluding one salt after another. A more favorable opportunity to do this would be afforded by a salt medium in which duplication of radicals had been avoided, provided the physiological necessities of the animal should be compatible with it. For several reasons it seemed to me much better to begin with single salts and proceed step by step to the more complex combinations. Hence each experiment of the following series is based upon the more or less tentative conclusions drawn from those which precede it. General and conclusive results can be shown only from the series as a whole.

Expt., Jan. 8, 1903.

The Stentors used in this experiment were lighter in color than hardy Stentors, and were somewhat undersized. Of the chemical conditions in the combination $Na_2HPO_4 + CaCl_2$, it is for our purpose necessary to know only that all the ions, or radicals, used are represented in the solution. $CaHPO_4$ is soluble in dilute solution and still more so in the presence of some other salts, for example NaCl (Dammer, '94, Band II. Theil 2, p. 320).

Media.	0 hr.	1 da.	2 da.	4 da.
.00050 m. Na_2HPO_4	50	50	50	50
.00050 m. $CaCl_2$	60	62	64	64
.00025 m. Na_2HPO_4 and .00025 m. $CaCl_2$	50	52	48	45
.00025 m. Ca2OH	50	30	33	31
.00050 m. Ca2OH	50	0		
.00050 m. HCl	50	0		

This experiment permits the following *tentative* conclusions: (1) Stentor is well adjusted to Na_2HPO_4 and to $CaCl_2$. (2) The low results of the combination $Na_2HPO_4 + CaCl_2$ were due to a sudden loss in one set of ten out of five such sets (50 Stentors). We may therefore infer from the uniformity of the other four sets, that Stentor is also well adjusted to this combination. Subsequent experiments uphold this view. (3) Stentor is not at all adjusted to excess of acid or alkali.

Expt., Jan. 9, 1903.

Stentors good, but slightly pale. Chemical conditions of solution like those of di-sodic phosphate + calcic chloride solution.

Medium.	0 hr.	1 da.	3 da.	4 da.	8 da.
00050 m. $CaCl_2$ and .00050 m. K_2HPO_4	50	57	57	59	59

Thus far the result indicates that Stentor is equally well adjusted to di-potassic phosphate and to di-sodic phosphate, when either is in combination with calcic chloride.

Expt., Jan. 14, 1903.

The Stentors used in this experiment were pale and not well fed.

Media.	0 da.	1 da.
.00100 m. $CaCl_2$	50	47
.00100 m. KCl	50	7
.00100 m. KNO_3	50	1
.00100 m. $MgSO_4$	50	10
.00100 m. K_2HPO_4	50	10

Of this list of physiological salts used *singly* and in *hypisotonic* concentration calcic chloride shows a mean result near unity and stands in marked contrast to the low values for the other salts. This effect is in harmony with the curves of the preceding section, where the same comparative results appear. It suggests the use of calcic chloride as a basis of comparison between different experiments when it occurs as a common factor in each. It gives a measure of the metabolic pitch characterizing the experiment.

Expt., Jan. 16, 1903.

Stentors are pale.

Media.	0 hr.	1 da.	7 da.
.00100 m. $CaCl_2$	50	30	
.00100 m. K_2HPO_4	50	0	
.00050 m. $CaCl_2$ and .00050 m. K_2HPO_4	50	48	34

Using calcic chloride as a standard, we should say that the Stentors here used present a low metabolic pitch. In view of this the higher mean result, and especially the longevity shown by the combination, strongly indicate that *Stentor is better adjusted to a combination of physiological salts than to any single salt of that combination*, provided that the *constitutive proportions* of the salts be observed. This is an important proposition for my subsequent discussion.

Expts., Jan. 17 and 22, 1903.

Media.	0 hr.	1-2 da.
JANUARY 17.		
.00100 m. $CaCl_2$	50	29
.00080 m. $CaCl_2$ and .00020 m. $MgSO_4$	50	32
JANUARY 22.		
.00100 m. $CaCl_2$	50	33
.00075 m. $CaCl_2$ and .00025 m. $MgSO_4$	50	35
.00050 m. $CaCl_2$ and .00025 m. K_2HPO_4 and .00025 m. $MgSO_4$	50	60

The animals at the end of this last experiment were healthier in color than at the end of the following experiment:

.00075 m. $CaCl_2$ and .00025 m. K_2HPO_4	50	54

I have ventured to record together the experiments of January 17 and 22 on the basis of their nearly equal results with calcic chloride.

The combination of the largest number of physiological salts used has given the best result, and there is additional evidence of the *good adjustment of Stentor to calcic chloride and di-potassic phosphate as is shown by the following experiments:*

Expt., Jan. 28, 1903.

Media.	0 hr.	1 da.	3 da.
.00055 m. $CaCl_2$ and .00015 m. $MgSO_4$ and .00015 m. $NaNO_3$ and .00015 m. K_2HPO_4	50	77	85
.00055 m. $CaCl_2$ and .00045 m. K_2HPO_4	50	76	83

This experiment merely adds evidence for the good adjustment to a combination. To test further the proposition regarding the comparative action of salts singly and in combination, I selected the above mixture of four salts for study in the next experiment. The high results obtained indicate that it represents constitutive proportions and is therefore adapted to the proposed test.

Expt., Feb. 2, 1903.

Media.	0 hr.	1 da.
.00055 m. $CaCl_2$ and .00015 m. $MgSO_4$ and .00015 m. $NaNO_3$ and .00015 m. K_2HPO_4	50	56
.00055 m. $CaCl_2$	50	26
.00015 m. $MgSO_4$	50	27
.00015 m. $NaNO_3$	50	4
.00045 m. K_2HPO_4	50	3
.00015 m. $MgSO_4$ and .00015 m. $NaNO_3$	50	37

The results of the experiment show (1) the excellent adjustment of Stentor to the four-salt mixture; (2) the superiority of this combination to any of its constituents used singly, it being double that of the metabolic pitch indicated by calcic chloride.

From the preceding experiments taken together inference of considerable reliability can be drawn as to the relative importance, or share of activity, of each of the constituents. The result of making partial combinations like the mixture of magnesic sulphate with sodic nitrate, as shown above, gives information on this point, and this method is further applied in the following final experiment.

Expt., Feb. 6, 1903.

Media.	0 hr.	3.5 hr.	2 da.
Distilled Water . . .	50	0	
.00055 m. $CaCl_2$ and .00015 m. $NaNO_3$ and .00015 m. $MgSO_4$ and .00015 m. K_2HPO_4	50	(all had disintegrated.)	65 (+16 dead).
.00055 m. $CaCl_2$ and .00015 m. $NaNO_3$	50		13
.00055 m. $CaCl_2$ and .00015 m. $MgSO_4$	50		33
.00055 m. $CaCl_2$ and .00015 m. K_2HPO_4	50		46 (+10 dead).

The results show, as before, the excellent adjustment of Stentor to the four-salt medium. They further show that the adjustment to two-salt media is best with calcic chloride + di-potassic phosphate; but it is also evident that the other constituents aided in producing the still better result of the four-salt medium.

I have used the above four-salt medium successfully for the establishment of mass-cultures. In such cases, owing to the addition of dry leaves or reeds for food, I have doubled or trebled the proportions in one hundred thousand, in order to overbalance the unknown salts introduced by the food material.

This four-salt medium represents at least one set of combinations to which Stentor is well adjusted, but it may not be the only useful combination of this description. However, numerous trials in making departures from the qualitative or quantitative constitution of this medium indicated, by their frequent failure, *that both the qualitative and quantitative range of substances to which Stentor is adjusted, is comparatively limited.* It would be of interest to have corresponding media well adjusted to other Protozoa tried with Stentor for the purpose of comparing the results. The empirical determination of a successful combination for Stentor we may now dismiss, and proceed to the consideration of some general aspects of the employment of single and of combined salts.

VIII. General Discussion of Single and Combined Salts.

The preceding experiments taken as a whole show that a medium consisting of several salts is more favorable to the physiological processes of the animal than one containing only a single salt. The physico-chemical phenomena underlying this fact seem to me to be the following. The experiments being made at hypisotonic concentrations, there were lower partial pressures outside the cell for all its contained salts except, probably, the one present in the medium. For this one salt there was a lower partial pressure within the cell as soon as the concentration applied outside exceeded the normally low concentration within. The low ash-content thus far obtained in all analyses of protoplasm shows that the normal concentration of individual salts within the cell is low. It is probably so low that most of the hypisotonic concentrations used in the preceding experiments exceeded the physiological limit for a single salt. When the contrary condition obtains, we approach, more and more as the concentration lowers, the conditions shown by cells in distilled water. In distilled water, as previously described, loss of salts brought about by constitutional alteration of the cell-wall explains the result. But under the conditions more probably existing in the above experiments we should have, in consequence of differences in partial pressures, two opposite movements of salts. Assuming permeability, the one salt applied would penetrate the cell and the other physiological salts would pass into the external medium. We are unable to determine how much of the effect to assign to each of the two processes, but they both contribute to the disturbance of the normal proportions of physiological salts. *They inhibit the division-reaction in Stentors by altering the constitutive proportion of its salts.* When the number of salts applied externally increases, the tendency to disproportion diminishes in consequence of fewer unbal-

anced pressures, and the results, as shown by division, are better. This is especially true if the normal relative proportions of the salts applied be preserved, as the four-salt medium probably does approximately. Changes in water-content within the limits here employed are not of essential or even perceptible influence upon metabolism. That under the prevailing conditions this factor is not important for our reckoning appears from a consideration of its ineffective magnitude at the extremes of the concentration curve. Stentors in distilled water did not suffer from excessive inward osmosis of water, nor did Stentors near the opposite extreme in milk sugar of hyperisotonic concentration suffer from excessive abstraction of water. If the movement of water at either extreme did not occur in sufficient intensity to prove harmful physiologically, such effect may of course be excluded from the intermediate ranges.

We may now attempt to describe in summary the physico-chemical processes for all concentrations of physiological salts. The form of the general curve obtained from the results expressing division-reactions shows a range of elevation more or less broad for medium concentrations, with depressions on either side which reach zero at extremely high or extremely low concentrations. At extremely high osmotic concentration of an indifferent substance (milk sugar) the destructive process is abstraction of water. At extremely low concentration (distilled water) the efficient factor in the harmful result is abstraction of physiological salts. For intermediate concentrations the osmotic introduction or abstraction of water, as the case may be, is a factor necessarily present, but of insufficient intensity to produce a disturbing physiological effect. Nor does simple osmotic redistribution of salts, that is, physical alteration of pressures of osmotically active particles, account for the result. At equal osmotic pressures, as curves previously given show, effects differ according to the individual salt. In the intermediate range of the concentration curve alteration of *constitutive proportion* is the factor of primary efficiency. It is worth while to notice that, owing to unbalanced partial pressures, the phenomena in hypisotonic and hyperisotonic concentrations are essentially similar processes. These consist in equilibration of pressures leading to the same result, — the alteration of the normal constitutive proportions of the physiological salts. Taking into account, then, the whole range of the general curve, there may be distinguished three classes of efficient factors. *At one extreme there is* (1) *abstraction of water; at the other,* (2) *abstraction of all permeating salts, probably in the relative proportion in which they are present in the organism; and in the intermediate range,* (3) *alteration of constitutive proportion.* Finally we must

recognize in *the permeability of the cell-membrane* or the protoplasmic alveoli a fundamentally important, but thus far often undetermined, factor. Without the determination of this factor physico-chemical explanation cannot proceed far.

These considerations lead to the following conception of the functions of the salts normally occurring in cells. The activity of the organism, in so far as it is dependent upon the physiological salts, depends, first of all, upon the qualitative nature of the constituent salts, that is, the function of these salts is primarily *constitutive*. In the activity of the organism they do service by virtue of their native qualities. If the organism had a different normal composition it would no longer exhibit the same reaction (physiological, chemical, physical) by which it now preserves its identity and individuality.

Secondly, the reactions of the organism depend, so far as they depend at all upon the physiological salts, upon the relative *proportions* of these to one another. Their function is quantitative as well as constitutive. A change in these proportions outside the normal limits of variation, would result, if successful, in loss of identity, if unsuccessful, in disease or death.

This theory by no means asserts that the salt which we know as a compound must exist as such in the organism. But the modified qualities, or energies of the substances contribute to and determine the activities of the organism, by virtue of their constitutive and quantitative relations to it.

From one point of view, at least, the organism is an organization of various *substances*, and the word organization means neither more nor less than correlation. If we choose to take the most advanced physico-chemical point of view, we may describe an organism as a specific correlation of energies.

The principles of constitutive and quantitative functions are nearly axiomatic. With less certainty, but yet with a large degree of probability, we can make a third proposition, which is derived from a consideration of the facts of adjustment. What indication do these give of the internal constitution of the cell? *The external medium to which the cell is normally adjusted represents more or less closely the internal qualitative and quantitative constitution of the cell.* This statement does not necessarily apply to the external medium of the whole plant or animal body, but it does apply to the liquid media in which the majority of cells pass their entire existence. Further, the proposition is not intended to exclude the possible presence in the medium of substances to which the cell is approximately indifferent.

The evidence for the above view of adjustment is mostly indirect. Where direct chemical analysis can give information of the internal constitution of any particular protoplasm, the method of determining adjustments would not be resorted to for that end. But where analysis cannot be applied, or where the results of analysis give too little indication of the original active form of combination of the radicals found by that process, the method of adjustments has considerable value. It is not of value because of its accuracy, for it is only a method of approximation, but it affords a wider physiological knowledge of substance-relations than can be obtained by analysis alone.

From the nature of the case we can adduce evidence of only a general and probable character for the view of adjustment above outlined. In our experiments we found Stentor well adjusted to certain calcium compounds and to certain phosphates. Other salts normally found in various kinds of protoplasm, if added, improved the degree of adjustment. It is probable that, had the experiments been longer continued the need of these latter salts, though present in small proportion, would have become more evident. All the salts used in the experiments upon adjustment are found in the list of necessary salts as determined by various feeding experiments, and also in the list of general protoplasmic constituents as determined by chemical analysis. Further general evidence comes from the consideration that the liquid medium is the sole source of income and the sole recipient of the outgo of the metabolic substances of the cell. Under the circumstances a general correspondence between medium and cell constitution could scarcely fail to exist, especially in the case of the physiological salts. That the metabolic activity of the organism is a metamorphosing process is not inconsistent with the above view.

In the case of Stentor an interesting ecological problem suggests itself in this connection. What relation is there between the adjustments which the animal has shown in the laboratory and the conditions in regard to substances which prevail in the natural homes of Stentor in the field? I have no data to present upon this subject, although it would be practicable to obtain such in this vicinity.

I now turn to a brief consideration of the question, What relation does the process of division in Stentor bear to the physiological salts? The substances experimented with fall into two classes. In the one stands solely potassic chloride. Of all the substances tried, it alone seemed to have the power of specifically disturbing the process of division. In what manner this was effected we are unable to say. All the

other substances stand in another class, whose activity in division I attribute primarily and principally to their share in metabolism. The manner of destruction of the cell (disintegration, for example) indicates a fundamental metabolic disturbance. Moreover, such divisions as occur seem to be normal. Culture experiments also show that when food is abundant, that is when metabolic conditions are good, division is increased, and *vice versa*, without anything peculiar appearing in the division process itself. The absence of any perceptible modification of the normal process of division, and, under given conditions, a fairly constant deviation of *rate* of division from the normal, are evidence of a primarily metabolic influence of these salts. However, it is true that calcic chloride and sodic chloride, for example, applied under the same conditions affect the rate of division in different degrees. Numerical differences that are fairly constant for a given metabolic condition of the animals result from the application, for example, of calcic chloride and of sodic chloride. From this fact it appears that these differences result from specific qualities of the salts. It is possible, and indeed probable, that these peculiarities extend even to those metabolisms which underlie the process of division.

IX. The Permeability of Stentor.

A few experiments were made upon permeability in Stentor. In one series the change in conductivity occurring in a salt medium containing the animals was measured. In the other series a volumetric determination of the chemical change resulting under similar conditions was made. I have records of eight experiments upon conductivity and of four chemical estimations. As all the experiments of a series showed the same essential results only a few examples will be described.

Expt., Oct. 14, 1902.

The test-cell used for the measurement of conductivity consisted of a beaker, 2 cm. in diameter and 8 cm. high, a thermometer with bulb immersed, and unplatinized electrodes 25 mm. square standing at a distance of 10 mm. apart in the fluid of the beaker. The latter contained in both test and control experiments 5 cc. of a salt medium, the composition of which will be given presently. Stentors were placed in the liquid in the test experiment only. The test-cell was connected in parallel with the measuring tube W″. The first column of the following record shows the number of the observation; the third gives the temperature read to within 0.05° C; the fourth gives the position of the

electrode in the measuring tube W″ as read directly against a millimeter scale placed behind it. The fifth column shows the position of the electrode as determined by means of the disk placed upon W″. The latter reading is accurate to within 0.02 mm. The physical relations of the apparatus are such that an increased reading in the position of the electrode is due to increased conductivity through the test-cell, and *vice versa*, when the latter is connected in *parallel* with the measuring tube. The absolute values of the conductivity would not add to the significance of the experiment and are not determined. Without the presence of Stentor in the test-cell the following record was obtained.

Observ.	Time.	Temp.	Scale.	Disk.
Wp 1	9.47	19.60	547.5	.222
Wp 2	9.50	19.60	547.5	.222
Wp 3	10.20	19.60	547.5	.222
Wp 4	10.33	10.60	547.5	.222
Wp 5	10.36	19.60	547.5	.229
Wp 6	10.47	19.60	547.5	.250
			Difference = +	.028 mm.

This test shows that the conditions in the test-cell are nearly constant for a period of one hour.

Corresponding measurements, made under the same conditions, except that the 5 cc. of the medium contained about fifty Stentors, gave the following record.

Observ.	Time.	Temp.	Scale.	Disk.
Wp 1	7.20	21.00	547.0	.125
Wp 2	7.26	21.00	547.0	.159
Wp 3	7.40	21.00	547.2	.222
Wp 4	7.49	21.00	547.3	.298
Wp 5	8.00	21.00	547.3	.375
Wp 6	8.06	21.00	547.3	.417
Wp 7	8.11	21.00	547.35	.437
Wp 8	8.21	21.00	547.4	.468
	1 hr.	0.00		.343 mm.

After the experiment fifty-two live Stentors in apparently good condition were recovered. Only one dead animal was observed.

The results show that in a period of one hour the conductivity of 5 cc. of this salt medium containing about fifty Stentors had increased by an amount far above the limit of error of the measurement.

The medium here used consisted of the following salts dissolved in ordinary distilled water.

Na_2HPO_4	.00030 m.
KNO_3	.00010 m.
Fe_2Cl_6	trace

In this case the concentration would of course be much higher. Determinations, as made in the next experiment, of the concentration of mass-cultures gave a range equivalent to that of a 0.00100 m. to 0.00200 m. calcic chloride solution. Without doubt Stentors taken from any of my mass-cultures and placed in the above test-medium made with distilled water would suffer a change from higher osmotic concentration to lower, that is, this medium presents for them hypisotonic conditions. Within the cell there exist unbalanced partial pressures of such physiological salts or ions as are not represented by the medium. In the latter di-sodic phosphate is *probably* present in a concentration that exceeds the amount of the same salt normally contained within the cell. In consequence of the unbalanced partial pressures on both sides there ensues a double movement of salts or ions, if the membrane is permeable to them. Since the medium is hypisotonic to the cell, there is also an inward movement of water tending to produce an increase of volume in the cells. The total result of all these processes is to increase the conductivity of the medium. *This result signifies an increased concentration of salts.* It does not follow directly that the membrane is permeable to the salts in question. The case is different from that of pure distilled water, where absorption of water from the medium by the cell could not in itself increase the conductivity of the medium. No possible loss from pure water could affect the conductivity of the remainder, whereas any outward movement whatever of salts would result in increased conductivity. But if in the present experiment, conductivity having increased, it could be shown that the cells exhibited no change in volume, then permeability would be demonstrated. Or if from the increase in concentration of the medium there were subtracted a correction, expressing the concentration-equivalent due to loss of water from the medium to the cell, a remainder, if any, would measure the permeability. Such a correction, determined by the haematokrit method, has been applied to chemical estimations in the case of blood corpuscles. I have not attempted this method with Stentor, and to find a concentration-equivalent in terms of conductivity may not be feasible. Hence the

above experiment is inconclusive. It is not probable, however, that the inward movement of water alone accounts for all the increase in conductivity. The probabilities are in favor of the permeability of the cell, for it is not likely to possess a membrane permeable to water alone.

Upon the whole, the method by measuring conductivity is too general to yield specific information upon permeability, because conductivity is influenced by too many factors, and is affected by various salts. Moreover, as Hamburger (:02, pp. 255, 259) has pointed out, under certain conditions some exchange of ions between medium and cell could take place without change in the conductivity of the former.

An experiment similar to the above was attempted upon Paramaecia, but these animals disintegrated under the process. Furthermore, two experiments were made upon *Spirostomum ambiguum.* In the first experiment the animals were placed in the same hypisotonic medium of di-sodic phosphate + potassic nitrate as that which was used for Stentor. The result showed a similar increase in conductivity. In the second experiment the test-medium consisted of native culture liquid that had been filtered through a washed filter and had then been well shaken for the purpose of aeration. This was practically an isotonic medium. *In a period of* 1.7 *hours no increase in conductivity was detected.* This result has two important bearings. It indicates the correctness of the view above expressed that increased conductivity in the hypisotonic medium was due to osmotic redistribution of salts and water. It furthermore indicates that the respiratory activity of the animal, though constant, is not of such quantitative or possibly qualitative nature as to increase the conductivity of the medium within a short period of time. The motive for making this experiment was to test the possibility that respiration by the cell as a whole, or excretion by the contractile vacuole, might result in the accumulation in the medium of measurable ionized products. The quantity of these should increase continuously and uniformly, and its determination by the measurement of conductivity would have been a valuable method for the investigation of metabolism in Protozoa. Various determinations upon isotonic media containing Stentors for longer periods than above described were made, but the results were inconclusive.

Expt., Dec. 13, 1902.

The object of this experiment was to determine the permeability of Stentor to chlorine. The method consisted in the estimation of the change in the content of chlorine and in the degree of alkalinity of a medium containing Stentors under isotonic conditions. This method is

due to Hamburger, and was used successfully by him for the demonstration of permeability in blood corpuscles.

In the selection of a medium two considerations were important. First, the medium must be favorable to the physiological processes of the animal. Previous experiments indicate that of all one-salt media calcic chloride would best meet the requirement. Secondly, the solution of calcic chloride must be as nearly as possible isotonic with the native culture medium from which the animals originate. How to obtain the osmotic value of culture liquids was a question of some difficulty. From conductivity measurements it was possible to infer that their salt-content was so low as to render most methods uncertain in result. After some consideration the following approximate, but for our purpose sufficiently accurate, method was adopted. It was determined what concentration of calcic chloride had the same conductivity as the culture liquid, and the former was then regarded as the osmotic equivalent of the culture medium. When the culture was started it had the following composition, the salt being dissolved in tap-water.

Na_2HPO_4	.00050 m.
KNO_3	.00005 m.
KCl	.00005 m.
$FeCl_3$ calculated	.000005 m.

To this had been added some hay, some leaves, and some aqueous extract of barley. The effect of these additions is to make the medium consist of a *miscellaneous mixture of physiological salts.* Exactly which of these are present and in what proportions is of course unknown. The method by measuring the depression of the freezing point is especially applicable in such cases, but here this value would have been too small to be reliable. As is well known, the coefficient of dissociation, i, of a salt solution in relation to its equivalent conductivity, Δ, is expressed by the equation $i = 1 + (k - 1)\,\alpha$ and $\alpha = \frac{\Delta_v}{l_K + l_A}$. In these formulae i is the coefficient of dissociation, k is the number of ions into which the salt splits, α is the coefficient of activity of Arrhenius, Δ_v is the equivalent conductivity at the concentration v, l_K and l_A are the velocities respectively of the kation K and anion A. But α may be found with sufficient accuracy for our purpose by taking the ratio of Δ at the given concentration, represented by Δ_v, to Δ at or nearly at infinite dilution, represented by Δ_∞, that is $\alpha = \frac{\Delta_v}{\Delta_\infty}$. For a number of physiological salts the

ratio $\frac{\Delta_v}{\Delta_\infty}$, taken from a table of conductivities to the nearest integer for $\Delta_v = .002$ n, is as follows: $CaCl_2 = 110/115$, $Na_2HPO_4 = 100/126$, $NaH_2PO_4 = 84/126$, $KNO_3 = 122/125$, $NaNO_3 = 101/104$, $KCl = 127/130$, $NaCl = 107/110$, $Na_2SO_4 = 105/110$, $K_2SO_4 = 126/133$. This ratio has the approximate value of 1. For univalent salts, the number of ions, κ, into which a molecule of salts dissociates $= 2$; for bivalent salts $= 3$, for the above phosphates $= 4$. Hence $i = 1 + (k - 1)\ a = 2, 3, 4$. In other words, the osmotic concentrations of those salts at .002 n. is equivalent to 2, 3, 4 times that of an undissociated salt. Hence if it be desired to take any one of the physiological salts to represent the osmotic value of a mixture of all of them, one whose value for $i = 3$ is probably the best choice. Partly for this reason and partly for the sake of good physiological adjustment I selected calcic chloride. Its degree of dissociation is near the average of that of all the salts in question, provided none of them is present in excessively large proportion. A concentration of calcic chloride having the same conductivity as that of the culture medium of the Stentors of this experiment may hence be regarded as *approximately* isotonic with the medium. Conductivity determinations were made with the test-cell previously described connected in parallel with the measuring tube W'', upon which the dial was used. The conductivity of the culture liquid from which the Stentors were to be taken was then measured, that is, the position of the electrodes in W'', as indicated by the pointer on the dial, was noted. It was not necessary to know the absolute value. Successive equal quantities of calcic chloride solutions of different concentrations were then introduced into the test-cell and the position of the electrode noted as before. After two calcic chloride solutions had been measured in this way the concentration for the third trial was found by interpolation from the first two. It developed incidentally that under the conditions of the apparatus then prevailing the sensitiveness of the instrument was $= .0001$ n. calcic chloride solution. *It was found that a .0022 n., that is a .0011 m. calcic chloride solution had the same conductivity as the culture medium.* Of course the same result could be obtained with fewer measurements and more calculation, but since conductivity measurements are made with certainty and rapidity, this method seemed the better.

Several hundred Stentors were introduced into 200 cc. of .0011 m. calcic chloride solution. The liquid was contained in a narrow graduate and the animals settled upon the bottom. A glass siphon of narrow

bore was placed in the graduate for the purpose of withdrawing portions of the liquid. Its inner end was bent upward, so that the process would not disturb the Stentors lying below. Its outer end was closed by a piece of rubber tubing with a clamp placed upon it. Portions were withdrawn for chemical examination according to the following schedule:

Dec. 13.	1.49 P. M.	— Placed Stentor in $CaCl_2$ solution.				
	1.53 P. M.	— Withdrew about	35 cc.	Portion	No.	1.
	2.18 P. M.	— "	" 35	"	"	2.
	2.50 P. M.	— "	" 35	"	"	3.
Dec. 15.	11.00 A. M.	— "	" 45	"	"	4.

The several portions withdrawn were immediately estimated for chlorine by argentic nitrate with neutral potassic chromate for indicator.

The amount of acid or alkali necessary to produce reaction with methyl orange and phenolthalein was also found. The estimations with argentic nitrate gave the following result:

CHLORINE.

Portion.	Time (approximate).	
1	5 min.	5 cc. required 10.95 cc. of .01 n. $AgNO_3$.
2	30 min.	5 cc. required 10.55 cc. of .01 n. $AgNO_3$.
3	1 hr.	5 cc. required 10.60 cc. of .01 n. $AgNO_3$.
4	2 da.	5 cc. required 11.80 cc. of .01 n. $AgNO_3$.

These determinations were made by means of a 20 cc. burette graduated to .05 cc. The sensitiveness and accuracy of the process exceeded this amount of the argentic nitrate solution. The estimations for acidity and alkalinity were as follows:

Portion.	Time.	
1	5 min.	5 cc. required .15 cc. of .01 n. HCl, methyl orange. 5 cc. required .05 cc. of .01 n. NaOH, phenolthalein.
2	30 min.	5 cc. required .15 cc. of .01 n. HCl, methyl orange. 5 cc. required .05 cc. of .01 n. NaOH, phenolthalein.
3	1 hr.	5 cc. required .15 cc. of .01 n. HCl, methyl orange.
4	2 da.	5 cc. required .18 cc. of .01 n. HCl, methyl orange.

The result shows that within a period of one hour immediately following the transfer of Stentors *the concentration of chlorine in the medium decreases.* If the Stentors did not change their volume, the result would demonstrate the penetration of chlorine into the cell. In that case, conditions being isotonic, some other ion would pass outward into the

medium. In experiments on blood corpuscles Hamburger (:02) found that one CO_3 ion could, under nearly isotonic conditions, change position with two chlorine ions, the corpuscles being permeable. The evidence of this was the increased alkalinity of the medium, due to the substitution in it of alkali carbonate for alkali chloride. In the present experiment the tests for alkalinity have fallen out negatively. An insoluble carbonate of calcium may nevertheless have been formed. Perhaps also an uncertain result was to be expected unless the Stentors were previously treated with a stronger carbon dioxide solution than their native culture medium (Hamburger, :02, p. 258). Upon the other hand if the Stentors changed volume, — as they would probably do even in isotonic media (Hamburger, : 02, p. 259), — a correction would have to be applied to the concentration of chlorine as found by chemical estimation. If the Stentors swell by absorption of water, the concentration of chlorine in the medium would be increased thereby. It was, however, diminished. If change of volume is to account for this, we must assume a shrinkage in the Stentors. Since no haematokrit determination was made, we have no means of estimating this factor accurately. It remains to be found out by experiment whether this operation is practicable with these animals. But in the approximately isotonic solution of calcic chloride used it is improbable that an important amount of swelling or shrinkage occurred. The various experiments upon Stentor recorded in the course of this research indicate that the animal does not readily react by a change in volume to even considerable variations of osmotic pressure of the physiological salts. This implies a good degree of permeability. The decrease in chlorine found in the present experiment may, therefore, be attributed *mostly* to the inward permeation of that ion, but this is largely a matter of opinion. The experiments upon permeability had not reached a decisive point when this work was closed, but I shall continue work on this subject, and hope to publish results soon.

X. The Extrusion of Color in Stentor.

This phenomenon, though obscure, and bearing no known direct relation to any of the preceding work, will nevertheless be described briefly. In the application of reagents to Stentor it was observed that in some media a mass of blue-green matter was extruded by the cell. This occurred instantly upon transference to the new medium, or, in some cases, after the lapse of a minute. The animal swims away leaving the extruded mass or cloud behind. Occasionally the extruded mass was observed to adhere to, and to be trailed along with, the moving animal.

No evident injury resulted to the Stentor either immediately or remotely. The animal later divided in media that had produced this effect. However, those media which were found most favorable to the organism did not produce extrusion, although these media contained a proportion of certain substances which alone would have produced it. The effect seemed to be inhibited by other substances present, which were never observed to produce extrusion. Observation showed that the rejected matter might originate from any part of the surface of the Stentor, its boundary frequently forming a line parallel to the outline of the animal. The growth of this envelope in thickness could easily be observed. The extruded matter varied somewhat in consistency and depth of color. At times it was scarcely visible, and seemed to disappear in the surrounding water. In other cases it was a heavy deep-green mass. It always showed a more or less gelatinous consistency, so that it could be shaken as a whole by jarring the watch-glass containing it. Its nature and mode of production made it a difficult object to examine. I was unable to discover any structure in it, and I think it was a homogeneous mass.

The close analogy of this phenomenon with extrusion of coloring matter from blood corpuscles was evident. It led to the suggestion that there might be here a relation to osmotic conditions similar to that shown by the process of "laking" in blood. Had this been true, the same use as in the latter case might have been made of Stentor cells to arrive at an approximate estimation of the osmotic concentration of their medium. However, the results of tests which entirely disprove this view of the phenomenon are given in the following table. The first column gives the number of the experiment, the second indicates the substance, and the third gives the extremes of concentration in molecular parts at which and between which extrusion was observed, — E signifying that extrusion occurred, 0 that none was observed. A parenthesis encloses the nearest concentration at which non-extrusion was observed. The original record contains observations at many intermediate concentrations, but these are not given in the table.

No.	Substance.	Range molecular parts.
1.	KCl	E .155–.001 (.0001)
2.	NaCl	E .1–.001 (.0001)
3.	KNO_3	E .06–.0006 (.0001 ?)
4.	Na_2SO_4	E .2–.001 (.0001)
5.	$(NH_4)_2SO_4$	E .2–.001 (.0001)
6.	Na_2HPO_4	E

No.	Substance.	Range molecular parts.
7.	NaOH	E .022–.0001 (.00005)
8.	HCl	E .001–.0004 (.0001)
9.	Lactose	E .2–.04 (.02)
10.	Chloroform	E Sat. aq. sol.–.1 sat.
11.	$CaCl_2$	0 .193–.0001
12.	Ca2OH	0 .0144–.00014
13.	$CaSO_4$	0 Sat. aq. solution
14.	$MgSO_4$	0 .014

The extrusions with sodic sulphate are peculiarly dense (heavy), deep in color, and of strong consistency. That with chloroform has much the same character, and well illustrates the formation of an envelope of this matter around the cell. Most of the salts and the acids give lighter-colored and less consistent extrusions.

The addition of calcic chloride to sodic sulphate solutions raises the concentration of the latter required to produce extrusion, and in sufficient quantity prevents this effect of sodic sulphate altogether.

This result demonstrates clearly enough that this is not an osmotic phenomenon, like the laking of blood corpuscles with some salts. Even in the latter case certain reagents, for example ammonic chloride (Hamburger, : 02, p. 169), cause extrusion in any concentration. For Stentor the substances tested form a series, at one end of which practically any concentration results in extrusion, at the other no concentration is able to produce this result. The series expresses the animal's adjustment to the different substances in regard to this phenomenon. The extruded substance is of such nature that simple osmotic change does not cause its rejection from the cell. The conditions point to a chemical process. It is well known that different classes of proteids exhibit characteristic solubilities or insolubilities toward different salt solutions. By means of these they are separable from one another. In view of these facts the suggestion is ventured that the extruded coloring matter is a proteid separated from the living protoplasm by some salts, and not at all by others.

XI. General Summary.

1. In the growth and normal action of free-living cells, the salt content of the liquid medium in which they live is a most important factor.

2. In cultures of Infusoria the different stages in the development of a culture have each their characteristic animals. The fermentation of food material in the early stages of a culture prevents the growth of

Stentor at that time, owing to the acidity of the medium. When the initial fermentation has ceased the acidity diminishes and Stentors multiply.

3. Division in Stentor can be both accelerated and modified in character by the presence of an excess of potassic chloride in an otherwise normal medium. In Paramaecium an acceleration which is normal in character occurs under the influence of a small proportion of chloroform in an otherwise natural medium.

4. The effects on the division of Stentor produced by the chlorides of calcium, potassium, and magnesium are represented by the curves given on p. 473. Each curve taken by itself shows the effect of the factor of concentration. Secondary curves connecting experiments at the same osmotic concentration (see explanation of curves, p. 466) make an angle with the axis of abscissas; that is, they show that the salts have specific effects in addition to their osmotic activities. Milk sugar at concentrations equal to or greater than those used with the above four salts is harmless for Stentor. This fact shows that abstraction of water was not a prominent factor in the action of the four salts. Excessive proportion of a single salt was the destructive factor. Measurement of conductivity shows that distilled water kills Stentors by abstraction of *salts*. At extremely high concentrations sugar, as well as salts, kills cells, partly at least by abstraction of *water*.

In regions of the concentration curve extending between these extremes the effects of different concentrations do not differ sharply, as shown, for example, by the curve for calcic chloride. Hence the theoretical concentration curve for a single salt has a more or less broad elevation in its middle, or isotonic, region and descending limbs towards both hyperisotonic and hypisotonic extremes.

5. The permeability of the cell membrane is altered by distilled water, probably being increased by it. In milk sugar it probably remains normal. The effect of salts upon the permeability of the cell is a highly important factor in their action on free-living cells.

6. The curves on page 473 show different degrees of adjustment of Stentor to the chlorides of calcium, potassium, sodium, and magnesium in the order named.

7. A combination of *several* physiological salts results in a more favorable adjustment than a medium containing only *one*.

8. Stentor is well adjusted to a medium composed of calcic chloride + sodic nitrate + magnesic sulphate + di-potassic phosphate in given proportions.

9. The method employed for determining the adjustments of an organism gives an approximate physiological indication of the internal chemical organization of the cell.

10. The relation of physiological salts to the cell depends upon the "constitutive proportions" of the salts in the cell. The effects of these salts upon division are in general produced through their influence on metabolism, but with specific differences of intensity in each case. Potassic chloride, used alone, is the only one that has been observed to exert a qualitative effect upon division.

11. The cells of Stentor are probably permeable to the chlorine ions.

12. The extrusion of coloring matter by Stentor is not directly due to osmotic action.

13. The precipitation of the coloring matter by neutral salts suggests that it may be a proteid.

XII. Bibliography.

Dammer, O.

'94. Handbuch der anorganischen Chemie. Stuttgart. Bd. 2, Th. 2, xiii + 966 pp.

Forster, J.

'73. Versuche über die Bedeutung der Aschenbestandtheile in der Nahrung. Zeitschr. f. Biol., Bd. 9, pp. 297–380.

Hamburger, H. J.

:02. Osmotischer Druck und Ionenlehre in den medicinischen Wissenschaften. Zugleich Lehrbuch physikalisch-chemischer Methoden. Wiesbaden. J. F. Bergmann. Bd. 1, xiii + 539 pp.

Herbst, C.

'92. Experimentelle Untersuchungen über den Einfluss der veränderten chemischen Zusammensetzung des umgebenden Mediums auf die Entwicklung der Thiere. Theil I. Versuche an Seeigeleiern. Zeitschr. f. Wiss. Zool., Bd. 55, pp. 446–518. Taf. 19, 20.

Herbst, C.

'93. Experimentelle Untersuchungen, etc. Theil II. Weiteres über die morphologische Wirkung der Lithiumsalze und ihre theoretische Bedeutung. Mitth. Zool. Sta. Neapel., Bd. 11, pp. 136–220. Taf. 9, 10.

Herbst, C.

'96. Experimentelle Untersuchungen, etc. Theil III. Ueber das Ineinandergreifen von normaler Gastrulation und Lithiumentwicklung (Züchtung von Lithiumlarven mit Entodarm und Mund). Theil IV. Die formative Wirkung des Lithiums auf befruchtete Eier von Asterias glacialis. Theil V. Ueber die

Unterdrückung von Entwicklungsprocessen (Wirkung von Kalium rhodanatum und Natrium butyricum). Theil VI. Ueber den Einfluss einiger anderer organischer Salze. Arch. f. Entw.-Mech., Bd. 2, Heft. 4, pp. 455–516. Taf. 26–29.

Herbst, C.

'97. Ueber die zur Entwickelung der Seeigellarven nothwendigen anorganischen Stoffe, ihre Rolle und ihre Vertretbarkeit. Theil I. Die zur Entwickelung nothwendigen anorganischen Stoffe. Arch. f. Entw.-Mech., Bd. 5, Heft. 4, pp. 649–793. Taf. 12–14.

Herbst, C.

'98. Ueber zwei Fehlerquellen beim Nachweis der Unentbehrlichkeit von Phosphor und Eisen für die Entwickelung der Seeigellarven. Arch. f. Entw.-Mech., Bd. 7, Heft. 2, 3, pp. 486–510.

Herbst, C.

:00. Ueber das Auseinandergehen von Furchungs- und Gewebezellen im kalkfreien Medium. Arch. f. Entw.-Mech., Bd. 9, Heft. 3, pp. 424–463.

Hertwig, O. und R.

'87. Ueber den Befruchtungs- und Theilungsvorgang des thierischen Eies unter dem Einfluss äusserer Agentien. Jena. Zeitschr. f. Naturwiss., Bd. 20, Heft 1, pp. 128–241 ; Heft 2–3, pp. 477–510, Taf. 3–9.

Kohlrausch, F., und Holborn, L.

'98. Das Leitvermögen der Elektrolyte, insbesondere der Lösungen. Methoden, Resultate und chemische Anwendung. Leipzig. B. G. Teubner. xvi + 211 pp.

Korschelt, E., und Heider, K.

:02. Lehrbuch der vergleichenden Entwicklungsgeschlichte der wirbellosen Thiere. Allgemeiner Theil. Erste Lieferung. Jena. G. Fischer. x + 538 pp.

Loeb, J.

'99. On the Nature of the Process of Fertilization and the Artificial Production of Normal Larvæ (Plutei) from the Unfertilized Eggs of the Sea Urchin. Amer. Jour. Physiol., Vol. 3, pp. 135–138.

Maltby, M. E.

'95. Methode zur Bestimmung grosser elektrolytischer Widerstände. Zeitschr. f. physik. Chemie, Bd. 18, Heft. 1, pp. 133–158.

Minot, C. S.

:03. A Laboratory Text-Book of Embryology. P. Blakiston's Son & Co., Philadelphia. xvii + 17–380 pp.

Morgan, T. H.

'99. The Action of Salt-Solutions on the Unfertilized and Fertilized Eggs of Arbacia, and of Other Animals. Arch. f. Entw.-Mech., Bd. 8, Heft. 3, pp. 448–539. Taf. 7–10.

Nernst, W.

'94. Methode zur Bestimmung von Dielektricitätskonstanten. Zeitschr. f. physik. Chemie, Band 14, Heft 4, pp. 622–663.

Peters, A. W.

:01. Some Methods for Use in the Study of Infusoria. Amer. Nat., Vol. 35, No. 415, pp. 553–559.

Pfeffer, W.

:00. The Physiology of Plants. A Treatise upon the Metabolism and Sources of Energy in Plants. 2d ed. (Translation by A. J. Ewart.) Vol. I. Oxford. xii + 632 pp.

Rywosch, D.

:00. Ueber die Bedeutung der Salze für das Leben der Organismen. Biol. Centralbl., Bd. 20, No. 12, pp. 413–421.

Schäfer, E. A.

'98. Text-Book of Physiology. New York. Macmillan. Vol. I. xviii + 1036 pp.

Sutton, F.

'96. A Systematic Handbook of Volumetric Analysis, etc. London. 7th ed. xi + 587 pp.

Tichomiroff, A.

'86. Die künstliche Parthenogenese bei Insecten. Arch. f. Physiol., Jahrg. 1886. Physiol. Abth., Suppl. Bd. pp. 35–36.

Tichomiroff, A.

'86[a]. Sullo sviluppo delle nova del bombice del gelso, etc. Boll. mens. Bachicoltura, Padova. s. II. a. III. No. 11–12.

Tichomiroff, A.

'88. Nochmals über Parthenogenesis bei Bombyx mori. Zool. Anz., Jahrg. 11, No. 282, pp. 342–344.

Viguier, C.

:01. Fécondation chimique ou Parthénogénèse ? Ann. Sci. Nat., Zool., sér. 8, tom. 12, pp. 87–138.

Winkler, H.

:00. Ueber die Furchung unbefruchteter Eier unter der Einwirkung von Extractivstoffen aus dem Sperma. Nachr. Gesellsch. Wissensch. Göttingen, Math.-physik. Kl., Jahrg. 1900, Heft 2. pp. 187–193.